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* * * * * * * * * *   Welcome to STN International   * * * * * * * * * *

 NEWS  1           Web Page URLs for STN Seminar Schedule - N. America
 NEWS  2           "Ask CAS" for self-help around the clock
 NEWS  3  SEP 09   CA/CAplus records now contain indexing from 1907 to the
                   present
 NEWS  4  DEC 08   INPADOC: Legal Status data reloaded
 NEWS  5  SEP 29   DISSABS now available on STN
 NEWS  6  OCT 10   PCTFULL: Two new display fields added
 NEWS  7  OCT 21   BIOSIS file reloaded and enhanced
 NEWS  8  OCT 28   BIOSIS file segment of TOXCENTER reloaded and enhanced
 NEWS  9  NOV 24   MSDS-CCOHS file reloaded
 NEWS 10  DEC 08   CABA reloaded with left truncation
 NEWS 11  DEC 08   IMS file names changed
 NEWS 12  DEC 09   Experimental property data collected by CAS now available
                   in REGISTRY
 NEWS 13  DEC 09   STN Entry Date available for display in REGISTRY and CA/CAplus
 NEWS 14  DEC 17   DGENE: Two new display fields added
 NEWS 15  DEC 18   BIOTECHNO no longer updated
 NEWS 16  DEC 19   CROPU no longer updated; subscriber discount no longer
                   available
 NEWS 17  DEC 22   Additional INPI reactions and pre-1907 documents added to CAS
                   databases
 NEWS 18  DEC 22   IFIPAT/IFIUDB/IFICDB reloaded with new data and search fields
 NEWS 19  DEC 22   ABI-INFORM now available on STN
 NEWS 20  JAN 27   Source of Registration (SR) information in REGISTRY updated
                   and searchable
 NEWS 21  JAN 27   A new search aid, the Company Name Thesaurus, available in
                   CA/CAplus
 NEWS 22  FEB 05   German (DE) application and patent publication number format
                   changes
 NEWS 23  MAR 03   MEDLINE and LMEDLINE reloaded
 NEWS 24  MAR 03   MEDLINE file segment of TOXCENTER reloaded
 NEWS 25  MAR 03   FRANCEPAT now available on STN

 NEWS EXPRESS  MARCH 5 CURRENT WINDOWS VERSION IS V7.00A, CURRENT
               MACINTOSH VERSION IS V6.0b(ENG) AND V6.0Jb(JP),
               AND CURRENT DISCOVER FILE IS DATED 3 MARCH 2004
 NEWS HOURS    STN Operating Hours Plus Help Desk Availability
 NEWS INTER    General Internet Information
 NEWS LOGIN    Welcome Banner and News Items
 NEWS PHONE    Direct Dial and Telecommunication Network Access to STN
 NEWS WWW      CAS World Wide Web Site (general information)

Enter NEWS followed by the item number or name to see news on that
specific topic.

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  agreement.  Please note that this agreement limits use to scientific
  research.  Use for software development or design or implementation
  of commercial gateways or other similar uses is prohibited and may
  result in loss of user privileges and other penalties.

  * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 22:23:33 ON 10 MAR 2004

=> file uspatful
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                     0.21       0.21

FILE 'USPATFULL' ENTERED AT 22:24:23 ON 10 MAR 2004
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FILE COVERS 1971 TO PATENT PUBLICATION DATE: 9 Mar 2004 (20040309/PD)
FILE LAST UPDATED: 9 Mar 2004 (20040309/ED)
HIGHEST GRANTED PATENT NUMBER: US6704933
HIGHEST APPLICATION PUBLICATION NUMBER: US2004045070
CA INDEXING IS CURRENT THROUGH 9 Mar 2004 (20040309/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 9 Mar 2004 (20040309/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: Dec 2003
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Dec 2003

>>> USPAT2 is now available. USPATFULL contains full text of the <<<
>>> original, i.e., the earliest published granted patents or <<<
>>> applications. USPAT2 contains full text of the latest US <<<
>>> publications, starting in 2001, for the inventions covered in <<<
>>> USPATFULL. A USPATFULL record contains not only the original <<<
>>> published document but also a list of any subsequent <<<
>>> publications. The publication number, patent kind code, and <<<
>>> publication date for all the US publications for an invention <<<
>>> are displayed in the PI (Patent Information) field of USPATFULL <<<
>>> records and may be searched in standard search fields, e.g., /PN, <<<
>>> /PK, etc. <<<

>>> USPATFULL and USPAT2 can be accessed and searched together <<<
>>> through the new cluster USPATALL. Type FILE USPATALL to <<<
>>> enter this cluster. <<<
>>> <<<
>>> Use USPATALL when searching terms such as patent assignees, <<<
>>> classifications, or claims, that may potentially change from <<<
>>> the earliest to the latest publication. <<<

This file contains CAS Registry Numbers for easy and accurate
substance identification.

```
=> e wada manabu/in
E1            1     WADA KYOJI/IN
E2           10     WADA MAKOTO/IN
E3            7 --> WADA MANABU/IN
E4            1     WADA MARI/IN
E5            2     WADA MASAE/IN
E6            1     WADA MASAFUMI/IN
E7           12     WADA MASAHARU/IN
E8           17     WADA MASAHIKO/IN
E9           57     WADA MASAHIRO/IN
E10           2     WADA MASAHUMI/IN
E11           1     WADA MASAKATSU/IN
E12           3     WADA MASAKAZU/IN

=> s e3
L1            7 "WADA MANABU"/IN

=> d l1,ti,1-7

L1   ANSWER 1 OF 7  USPATFULL on STN
TI     Anti-HIV agents

L1   ANSWER 2 OF 7  USPATFULL on STN
TI     Actin filament-binding protein "l-Afadin"

L1   ANSWER 3 OF 7  USPATFULL on STN
TI     Air cleaner device of engine

L1   ANSWER 4 OF 7  USPATFULL on STN
TI     Memory module using dram and method of refreshing the memory module

L1   ANSWER 5 OF 7  USPATFULL on STN
```

TI Mounting apparatus for automotive engines

L1 ANSWER 6 OF 7 USPATFULL on STN
TI Engine intake structure

L1 ANSWER 7 OF 7 USPATFULL on STN
TI Fuel supply system

=> d l1,cbib,ab

L1 ANSWER 1 OF 7 USPATFULL on STN
2002:337939 Anti-HIV agents.
    Wada, Manabu, Hyogo, JAPAN
    Wada, Naoko, Hyogo, JAPAN
    JCR Pharmaceuticals Co., Ltd., Hyogo, JAPAN, 659-0021 (non-U.S.
    corporation)
    US 2002193304 A1 20021219
    APPLICATION: US 2002-76421 A1 20020219 (10)
    PRIORITY: JP 2001-42655 20010220
    JP 2001-184284 20010619
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Anti-HIV agents are disclosed. The agents comprise as the active
    component one of ligand molecules that bind to CD87. Examples of such
    ligand molecules included the high molecular weight urokinase-type
    plasminogen activator, its amino-terminal fragment, their analogues and
    anti-CD87 antibodies.

=> e wada naoko/in
E1   1   WADA NAOHISA/IN
E2   8   WADA NAOKI/IN
E3   2 --> WADA NAOKO/IN
E4   1   WADA NAOTAKE/IN
E5   3   WADA NAOTO/IN
E6   2   WADA NAOYA/IN
E7   1   WADA NAOYUKI/IN
E8   1   WADA NARUO/IN
E9   4   WADA NOBORU/IN
E10   3   WADA NOBUAKI/IN
E11   19   WADA NOBUHIDE/IN
E12   1   WADA NOBUHITO/IN

=> s e3
L2   2 "WADA NAOKO"/IN

=> d l2,ti,1-2

L2 ANSWER 1 OF 2 USPATFULL on STN
TI Anti-HIV agents

L2 ANSWER 2 OF 2 USPATFULL on STN
TI Method for producing a polyfluoropropionyl halide

=> file medline
COST IN U.S. DOLLARS   SINCE FILE   TOTAL
                                          ENTRY   SESSION
FULL ESTIMATED COST   4.54   4.75

FILE 'MEDLINE' ENTERED AT 22:25:28 ON 10 MAR 2004

 FILE LAST UPDATED: 10 MAR 2004 (20040310/UP). FILE COVERS 1953 TO DATE.

 On February 29, 2004, the 2004 MeSH terms were loaded. See HELP RLOAD

for details.

MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the MeSH 2004 vocabulary. See http://www.nlm.nih.gov/mesh/ and http://www.nlm.nih.gov/pubs/techbull/nd03/nd03_mesh.html for a description of changes.

This file contains CAS Registry Numbers for easy and accurate substance identification.

```
=> e wada m/in
'IN' IS NOT A VALID EXPAND FIELD CODE FOR FILE 'MEDLINE'
The indicated field code is not available for EXPAND in this
file.  To see a list of valid EXPAND field codes, enter HELP
SFIELDS at an arrow prompt (=>).

=> e wada m/au
E1            1     WADA L L/AU
E2            1     WADA LESLIE/AU
E3          875 --> WADA M/AU
E4           13     WADA M L/AU
E5            1     WADA M L F/AU
E6            3     WADA M R/AU
E7            5     WADA M Y/AU
E8            1     WADA MAKI/AU
E9            4     WADA MAKOTO/AU
E10           9     WADA MANABU/AU
E11          12     WADA MASAHIRO/AU
E12           2     WADA MASAHISA/AU

=> s e3
L3          875 "WADA M"/AU

=> s l3 and (urokinase)
          9092 UROKINASE
L4           2 L3 AND (UROKINASE)

=> d l4,ti,1-2

L4   ANSWER 1 OF 2     MEDLINE on STN
TI   Amino-terminal fragment of urokinase-type plasminogen activator inhibits
     HIV-1 replication.

L4   ANSWER 2 OF 2     MEDLINE on STN
TI   Effect of ticlopidine and other antithrombotics on the venous thrombosis
     induced by endothelial damage of jugular vein in rats.


=> d l4,cbib,ab

L4   ANSWER 1 OF 2     MEDLINE on STN
2001327647.    PubMed ID: 11394884.   Amino-terminal fragment of
     urokinase-type plasminogen activator inhibits HIV-1 replication. Wada
     M; Wada N A; Shirono H; Taniguchi K; Tsuchie H; Koga J. (Laboratories for
     Bioengineering and Research, JCR Pharmaceuticals Company, Ltd., 2-2-10
     Murotani, Nishi-ku, Kobe, 651-2241, Japan.. wada-m@jcrpharm.co.jp) .
     Biochemical and biophysical research communications, (2001 Jun 8) 284 (2)
     346-51.  Journal code: 0372516. ISSN: 0006-291X. Pub. country: United
     States. Language: English.
AB   CD8+ T lymphocytes have been shown to produce unidentified soluble factors
     active in suppressing HIV-1 replication.  In this study, we purified an
     HIV-1 suppressing activity from the culture supernatant of an immortalized
     CD8+ T cell clone, derived from an HIV-1 infected long-term nonprogressor,
     and identified this activity as the amino-terminal fragment (ATF) of
     urokinase-type plasminogen activator (uPA).  ATF is catalytically
     inactive, but suppresses the release of viral particles from the HIV-1
```

infected cell lines via binding to its receptor CD67. In contrast, cell proliferation and the secretion of an HIV-1 LTR driven reporter gene product were not affected by ATF. These findings suggest that ATF may inhibit the assembly and budding of HIV-1, which provides a novel therapeutic strategy for AIDS.
Copyright 2001 Academic Press.

```
=> e wada n/au
E1          10      WADA MORIMASA/AU
E2           2      WADA MOTOSHI/AU
E3         226 --> WADA N/AU
E4           1      WADA N A/AU
E5           1      WADA NAKAGAWA C/AU
E6           1      WADA NANCY/AU
E7           1      WADA NAOHIRO/AU
E8           4      WADA NAOHISA/AU
E9           4      WADA NAOKI/AU
E10          3      WADA NAOKO/AU
E11          4      WADA NAOMI/AU
E12          1      WADA NAOSHI/AU

=> s e10
L5           3 "WADA NAOKO"/AU

=> d l5,ti,1-3

L5   ANSWER 1 OF 3     MEDLINE on STN
TI   Pyruvate dehydrogenase E1alpha subunit deficiency in a female patient:
     evidence of antenatal origin of brain damage and possible etiology of
     infantile spasms.

L5   ANSWER 2 OF 3     MEDLINE on STN
TI   Antitumor effect of arsenic trioxide in murine xenograft model.

L5   ANSWER 3 OF 3     MEDLINE on STN
TI   Amyloid deposition of systemic myeloma-associated amyloidosis excludes
     actinic elastotic material.


=> file uspatful
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                       2.50       7.25

FILE 'USPATFULL' ENTERED AT 22:28:52 ON 10 MAR 2004
CA INDEXING COPYRIGHT (C) 2004 AMERICAN CHEMICAL SOCIETY (ACS)

FILE COVERS 1971 TO PATENT PUBLICATION DATE: 9 Mar 2004 (20040309/PD)
FILE LAST UPDATED: 9 Mar 2004 (20040309/ED)
HIGHEST GRANTED PATENT NUMBER: US6704933
HIGHEST APPLICATION PUBLICATION NUMBER: US2004045070
CA INDEXING IS CURRENT THROUGH 9 Mar 2004 (20040309/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 9 Mar 2004 (20040309/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: Dec 2003
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Dec 2003

>>>  USPAT2 is now available.  USPATFULL contains full text of the     <<<
>>>  original, i.e., the earliest published granted patents or         <<<
>>>  applications.  USPAT2 contains full text of the latest US         <<<
>>>  publications, starting in 2001, for the inventions covered in     <<<
>>>  USPATFULL.  A USPATFULL record contains not only the original     <<<
>>>  published document but also a list of any subsequent              <<<
>>>  publications.  The publication number, patent kind code, and      <<<
>>>  publication date for all the US publications for an invention     <<<
>>>  are displayed in the PI (Patent Information) field of USPATFULL   <<<
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>>>  records and may be searched in standard search fields, e.g., /PN,  <<<
>>>  /PK, etc.                                                          <<<

>>>  USPATFULL and USPAT2 can be accessed and searched together         <<<
>>>  through the new cluster USPATALL.  Type FILE USPATALL to           <<<
>>>  enter this cluster.                                                <<<
>>>                                                                     <<<
>>>  Use USPATALL when searching terms such as patent assignees,        <<<
>>>  classifications, or claims, that may potentially change from      <<<
>>>  the earliest to the latest publication.                            <<<

This file contains CAS Registry Numbers for easy and accurate
substance identification.

=> s(urokinase)
S(UROKINASE) IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s urokinase
L6         6244 UROKINASE

=> s l6 and (human urokinase plasminogen activator)
         375362 HUMAN
           6244 UROKINASE
          11469 PLASMINOGEN
          45911 ACTIVATOR
             38 HUMAN UROKINASE PLASMINOGEN ACTIVATOR
                  (HUMAN(W)UROKINASE(W)PLASMINOGEN(W)ACTIVATOR)
L7           38 L6 AND (HUMAN UROKINASE PLASMINOGEN ACTIVATOR)

=> s l7 and (human urokinase plasminogen activator/clm)
          71920 HUMAN/CLM
            729 UROKINASE/CLM
           1280 PLASMINOGEN/CLM
           8215 ACTIVATOR/CLM
              2 HUMAN UROKINASE PLASMINOGEN ACTIVATOR/CLM
                  ((HUMAN(W)UROKINASE(W)PLASMINOGEN(W)ACTIVATOR)/CLM)
L8            2 L7 AND (HUMAN UROKINASE PLASMINOGEN ACTIVATOR/CLM)

=> d l8,cbib,1-2

L8   ANSWER 1 OF 2  USPATFULL on STN
2003:3043 Recombinant adenoviral vectors and their utility in the treatment of
    various types of fibrosis: hepatic, renal, pulmonary, as well as
    hypertrophic scars.
    Armendariz Borunda, Juan, Prado Coapa, MEXICO
    Aguilar Cordova, Estuardo, Col. Prado Coapa, MEXICO
    US 2003003077 A1 20030102
    APPLICATION: US 2002-98359  A1  20020318 (10)
    PRIORITY: MX 1999-998515  19990917
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L8   ANSWER 2 OF 2  USPATFULL on STN
97:71159 Peptide inhibitors of urokinase receptor activity.
    Rosenberg, Steven, Oakland, CA, United States
    Doyle, Michael V., Oakland, CA, United States
    Chiron Corporation, Emeryville, CA, United States (U.S. corporation)
    US 5656726 19970812
    APPLICATION: US 1995-370567  19950109 (8)
    DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
```

```
=> d his

     (FILE 'HOME' ENTERED AT 22:23:33 ON 10 MAR 2004)

     FILE 'USPATFULL' ENTERED AT 22:24:23 ON 10 MAR 2004
                E WADA MANABU/IN
L1            7 S E3
                E WADA NAOKO/IN
L2            2 S E3

     FILE 'MEDLINE' ENTERED AT 22:25:28 ON 10 MAR 2004
                E WADA M/AU
L3          875 S E3
L4            2 S L3 AND (UROKINASE)
                E WADA N/AU
L5            3 S E10

     FILE 'USPATFULL' ENTERED AT 22:28:52 ON 10 MAR 2004
L6         6244 S UROKINASE
L7           38 S L6 AND (HUMAN UROKINASE PLASMINOGEN ACTIVATOR)
L8            2 S L7 AND (HUMAN UROKINASE PLASMINOGEN ACTIVATOR/CLM)

=> s l7 not l8
L9           36 L7 NOT L8

=> d l9,cbib,1-36

L9   ANSWER 1 OF 36  USPATFULL on STN
2004:57950 Antisense modulation of urokinase plasminogen activator expression
    Baker, Brenda F., Carlsbad, CA, UNITED STATES
    Freier, Susan M., San Diego, CA, UNITED STATES
    Watt, Andrew T., Vista, CA, UNITED STATES
    US 2004043957 A1 20040304
    APPLICATION: US 2003-665216  A1  20030919 (10)
    DOCUMENT TYPE: Utility; APPLICATION.

L9   ANSWER 2 OF 36  USPATFULL on STN
2004:40522 Regulation of human skin healing.
    Herlyn, Meenhard, Wynnewood, PA, UNITED STATES
    Berking, Carola, Munich, GERMANY, FEDERAL REPUBLIC OF
    Satyamoorthy, Kapaettu, Santhekatte, INDIA
    Velazquez, Omaida, Cherry Hill, NJ, UNITED STATES
    US 2004031067 A1 20040212
    APPLICATION: US 2003-398980  A1  20030822 (10)
    WO 2001-US31555 20011011
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9   ANSWER 3 OF 36  USPATFULL on STN
2004:2116 Human tissue urokinase type plasminogen activator production.
    Hung, Paul Porwen, Bryn Mawr, PA, UNITED STATES
    Wu, Bryan T. H., Taipei, TAIWAN, PROVINCE OF CHINA
    Global Biotech, Inc. a Taiwan corporation (U.S. corporation)
    US 2004002137 A1 20040101
    APPLICATION: US 2003-401077  A1  20030327 (10)
    PRIORITY: US 2002-371013P  20020409 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9   ANSWER 4 OF 36  USPATFULL on STN
2003:324324 Abrogen polypeptides, nucleic acids encoding them and methods for
    using them to inhibit angiogenesis.
    Nesbit, Mark, Vincennes, FRANCE
    Fong, Timothy C., Moraga, CA, UNITED STATES
    Brockstedt, Dirk, Oakland, CA, UNITED STATES
```

US 2005220290 A1 20051211
APPLICATION: US 2002-233675 A1 20020904 (10)
PRIORITY: US 2001-316300P 20010904 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 5 OF 36 USPATFULL on STN
2003:285082 Adenovirus-mediated intratumoral delivery of an angiogenesis antagonist for the treatment of tumors.
Li, Hong, Epinay sur Seine, FRANCE
Lu, He, Epinay sur Seine, FRANCE
Griscelli, Frank, Paris, FRANCE
Opolon, Paule, Paris, FRANCE
Soria, Claudine, Taverny, FRANCE
Ragot, Thierry, Meudon, FRANCE
Legrand, Yves, Paris, FRANCE
Soria, Jeannette, Taverny, FRANCE
Mabilat, Christelle, Corbeil Essonnes, FRANCE
Perricaudet, Michel, Ecrosnes, FRANCE
Yeh, Patrice, Gif sur Yvette, FRANCE
Gencell SAS, Vitry sur Seine, FRANCE (non-U.S. corporation)
US 6638502 B1 20031028
WO 9849321 19981105
APPLICATION: US 2000-403736 20000629 (9)
WO 1998-EP2491 19980427
PRIORITY: US 1997-44980P 19970428 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 6 OF 36 USPATFULL on STN
2003:283096 Composition for the treatment of damaged tissue.
Dack, Kevin Neil, Kent, UNITED KINGDOM
Davies, Michael John, Kent, UNITED KINGDOM
Fish, Paul Vincent, Kent, UNITED KINGDOM
Huggins, Jonathan Paul, Kent, UNITED KINGDOM
McIntosh, Fraser Stuart, Kent, UNITED KINGDOM
Occleston, Nicholas Laurence, Kent, UNITED KINGDOM
Pfizer Inc. (non-U.S. corporation)
US 2003199440 A1 20031023
APPLICATION: US 2002-131985 A1 20020425 (10)
PRIORITY: GB 1999-30768 19991229
US 2000-186426P 20000302 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 7 OF 36 USPATFULL on STN
2003:165417 Formulations for pulmonary delivery.
Katyama, Derrick, Denver, CO, UNITED STATES
Manning, Mark C., Denver, CO, UNITED STATES
Stringer, Kathleen A., Denver, CO, UNITED STATES
Repine, John E., Englewood, CO, UNITED STATES
University Technology Corporation (U.S. corporation)
US 2003113271 A1 20030619
APPLICATION: US 2002-327476 A1 20021224 (10)
PRIORITY: US 1997-36566P 19970129 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 8 OF 36 USPATFULL on STN
2003:140913 Suppression of inhibitors.
Brunner, Niels, Virum, DENMARK
Romer, John, Copenhagen, DENMARK
Ellis, Vincent, Woodford Green, UNITED KINGDOM
Pyke, Charles, Copenhagen, DENMARK
Grondahl-Hansen, Jan, Holte, DENMARK
Pappot, Helle Pedersen, Allerod, DENMARK

Hansen, Heine Hol, Holte, DENMARK
Dano, Keld, Charlottenlund, DENMARK
US 2003096755 A1 20030522
APPLICATION: US 2003-336513 A1 20030102 (10)
PRIORITY: DK 1993-851 19930716
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 9 OF 36 USPATFULL on STN
2003:134663 Structure based discovery of inhibitors of matriptase for the treatment of cancer and other conditions.
Lin, Chen-Yong, Falls Church, VA, UNITED STATES
Dickson, Robert B., Kensington, MD, UNITED STATES
Wang, Shaomeng, Rockville, MD, UNITED STATES
Enyedy, Istvan, Mount Ranier, MD, UNITED STATES
Lee, Sheau-Ling, Falls Church, VA, UNITED STATES
US 2003092752 A1 20030515
APPLICATION: US 2001-885295 A1 20010621 (9)
PRIORITY: US 2000-213073P 20000621 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 10 OF 36 USPATFULL on STN
2003:112949 Human tissue **urokinase** type plasminogen activator formulation.
Hung, Paul Porwen, Bryn Mawr, PA, UNITED STATES
Chang, Kenneth S. S., Taipei, JAPAN
Wu, Bryan T. H., Taipei, JAPAN
Huang, Kuo-Kuei, Hsinchu, TAIWAN, PROVINCE OF CHINA
US 2003077682 A1 20030424
APPLICATION: US 2002-236881 A1 20020905 (10)
PRIORITY: US 2001-318173P 20010907 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 11 OF 36 USPATFULL on STN
2003:38334 **Urokinase**-type plasminogen activator receptor.
Dano, Keld, Charlottenlund, DENMARK
Blasi, Francesco, Charlottenlund, DENMARK
Roldan, Ann Louring, Vallensbaek, DENMARK
Cubellis, Maria Vittoria, Napoli, ITALY
Masucci, Maria Teresa, Napoli, ITALY
Appella, Ettore, Chevy Chase, MD, UNITED STATES
Schleuning, Wolf-Dieter, Berlin, GERMANY, FEDERAL REPUBLIC OF
Behrendt, Niels, Bagsvaerd, DENMARK
Ronne, Ebbe, Copenhagen, DENMARK
Kristensen, Peter, Copenhagen, DENMARK
Pollanen, Jari, Espoo, FINLAND
Salonen, Eeva-Marjatta, Espoo, FINLAND
Stephens, Ross W., Helsinki, FINLAND
Tapiovaara, Hannele, Helsinki, FINLAND
Vaheri, Antti, Kauniainen, FINLAND
Moller, Lisbeth Birk, Bagsvaerd, DENMARK
Ellis, Vincent, Copenhagen, DENMARK
Lund, Leif Roge, Copenhagen, DENMARK
Ploug, Michael, Copenhagen, DENMARK
Pyke, Charles, Soborg, DENMARK
Patthy, Laszlo, Budapest, HUNGARY
US 2003027981 A1 20030206
APPLICATION: US 2001-755109 A1 20010108 (9)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 12 OF 36 USPATFULL on STN
2002:322541 Method for increasing the serum half-life of a biologically active molecule.
Drummond, Robert J., Richmond, CA, UNITED STATES

Rosenberg, Steve, Oakland, CA, UNITED STATES
US 2002182705 A1 20021205
APPLICATION: US 2002-123092 A1 20020411 (10)
PRIORITY: US 1998-76964P 19980305 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 13 OF 36 USPATFULL on STN
2002:322027 Human liver progenitors.
Reid, Lola M., Chapel Hill, NC, UNITED STATES
Moss, Nicholas, Carrboro, NC, UNITED STATES
Kubota, Hiroshi, Chapel Hill, NC, UNITED STATES
US 2002182188 A1 20021205
APPLICATION: US 2000-487318 A1 20000119 (9)
PRIORITY: US 1999-116331P 19990119 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 14 OF 36 USPATFULL on STN
2002:287499 Diagnostic method.
Smith, John Craig, Macclesfield, UNITED KINGDOM
US 2002160362 A1 20021031
APPLICATION: US 2001-773599 A1 20010202 (9)
PRIORITY: GB 2000-2366 20000203
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 15 OF 36 USPATFULL on STN
2002:262445 UPAR mimicking peptide.
Blasi, Francesco, Milan, ITALY
Fazioli, Francesca, Ancona, ITALY
Resnati, Massimo, Milan, ITALY
Sidenius, Nicolai, Milan, ITALY
Fondazione Centro San Raffaele del Monte Tabor, Milan, ITALY (non-U.S. corporation)Universita Degli Studi di Milano, Milan, ITALY (non-U.S. corporation)
US 6462170 B1 20021008
WO 9842733 19981001
APPLICATION: US 1999-381244 19991020 (9)
WO 1998-EP1547 19980318 19991020 PCT 371 date
PRIORITY: US 1997-41112P 19970320 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 16 OF 36 USPATFULL on STN
2002:165190 METHOD FOR INCREASING THE SERUM HALF-LIFE OF A BIOLOGICALLY ACTIVE MOLECULE.
DRUMMOND, ROBERT, RICHMOND, CA, UNITED STATES
ROSENBERG, STEVE, OAKLAND, CA, UNITED STATES
US 2002086819 A1 20020704
APPLICATION: US 1999-263117 A1 19990305 (9)
PRIORITY: US 1998-76964P 19980305 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 17 OF 36 USPATFULL on STN
2002:119314 MONONUCLEAR PHAGOCYTES IN THERAPEUTIC DRUG DELIVERY.
LEWIS, CLAIRE E., SHEFFIELD, UNITED KINGDOM
HARRIS, ADRIAN L., OXFORD, UNITED KINGDOM
MARSHALL, JULIAN M, OXFORD, UNITED KINGDOM
US 2002061294 A1 20020523
APPLICATION: US 1999-284009 A1 19990405 (9)
WO 1997-GB2709 19971008
PRIORITY: GB 1996-20952 19961009
GB 1997-1975 19970130
GB 1997-3670 19970221

DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 18 OF 36 USPATFULL on STN
2002:72649 Liver tissue source.
Reid, Lola M., Chapel Hill, NC, UNITED STATES
Lecluyse, Edward L., Chapel Hill, NC, UNITED STATES
US 2002039786 A1 20020404
APPLICATION: US 2001-764359 A1 20010119 (9)
PRIORITY: US 2000-176798P 20000119 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 19 OF 36 USPATFULL on STN
2001:188694 Suppression of inhibitors.
Brunner, Niels, Virum, Denmark
Romer, John, Copenhagen, Denmark
Ellis, Vincent, Woodford Green, Great Britain
Pyke, Charles, Copenhagen, Denmark
Grondahl-Hansen, Jan, Holte, Denmark
Pappot, Helle Pedersen, Allerod, Denmark
Hansen, Heine Hoi, Holte, Denmark
Dano, Keld, Charlottenlund, Denmark
US 2001034327 A1 20011025
APPLICATION: US 2001-836323 A1 20010418 (9)
PRIORITY: DK 1993-851 19930716
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 20 OF 36 USPATFULL on STN
2001:93478 **Urokinase**-type plasminogen activator receptor.
Dan.o slashed. , Keld, Charlottenlund, Denmark
Blasi, Francesco, Charlottenlund, Denmark
Roldan, Ann Louring, Vallensb.ae butted.k, Denmark
Cubellis, Maria Vittoria, Naples, Italy
Masucci, Maria Teresa, Naples, Italy
Appella, Ettore, Chevy Chase, MD, United States
Schleuning, W.D., Berlin, Germany, Federal Republic of
Behrendt, Niels, Bagsv.ae butted.rd, Denmark
R.o slashed.nne, Ebbe, Copenhagen, Denmark
Kristensen, Peter, Copenhagen, Denmark
Pollanen, Jari, Espoo, Finland
Salonen, Eeva-Marjatta, Espoo, Finland
Stephens, Ross W., Vantaa, Finland
Tapiovaara, Hannele, Helsinki, Finland
Vaheri, Antti, Kauniainen, Finland
M.o slashed.ller, Lisbeth Birk, Bagsv.ae butted.rd, Denmark
Ellis, Vincent, Copenhagen, Denmark
Lund, Leif R.o slashed.ge, Copenhagen, Denmark
Ploug, Michael, Copenhagen, Denmark
Pyke, Charles, S.o slashed.borg, Denmark
Patthy, Laszlo, Budapest, Hungary
Cancerforskningsfondet af 1989, Denmark (non-U.S. corporation)
US 6248712 B1 20010619
APPLICATION: US 1995-442108 19950516 (8)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 21 OF 36 USPATFULL on STN
2001:63243 Suppression of inhibitors.
Brunner, Nils, Hellerup, Denmark
R.o slashed.mer, John, Copenhagen, Denmark
Ellis, Vincent, Woodford Green, United Kingdom
Pyke, Charles, Hiller.o slashed.d, Denmark
Gr.o slashed.ndahl-Hansen, Jan, Holte, Denmark
Pedersen, Helle, Aller.o slashed.d, Denmark

Hansen, Heine H.o slashed.j, Holte, Denmark
Dan.o slashed. , Keld, Charlottenlund, Denmark
Cancerforskningsfonden AF 1989, Copenhagen K, Denmark (non-U.S. corporation)
US 6224865 B1 20010501
WO 9502413 19950126
APPLICATION: US 1996-583129 19960515 (8)
WO 1994-DK288 19940718 19960515 PCT 371 date 19960515 PCT 102(e) date
PRIORITY: DK 1993-851 19930716
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 22 OF 36 USPATFULL on STN
2000:174914 Method of producing transgenic animals for xenotransplantation expressing both an enzyme masking or reducing the level of the gal epitope and a complement inhibitor.
Diamond, Lisa E., Princeton, NJ, United States
Logan, John S., Robbinsville, NJ, United States
Byrne, Geurard W., Allentown, NJ, United States
Sharma, Ajay, Lawrenceville, NJ, United States
Nextran Inc., Princeton, NJ, United States (U.S. corporation)
US 6166288 20001226
APPLICATION: US 1996-675773 19960703 (8)
PRIORITY: US 1995-4461P 19950927 (60)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 23 OF 36 USPATFULL on STN
2000:117278 Antibodies and their use.
Dan.o slashed., Keld, Charlottenlund, Denmark
R.o slashed.nne, Ebbe, Copenhagen, Denmark
Behrendt, Niels, Bagsvaerd, Denmark
Ellis, Vincent, Copenhagen, Denmark
H.o slashed.yer-Hansen, Gunilla, Gentofte, Denmark
Pyke, Charles, S.o slashed.borg, Denmark
Bruenner, Nils, Virum, Denmark
Cancerforskiningsfonden af 1989, Copenhagen, Denmark (non-U.S. corporation)
US 6113897 20000905
APPLICATION: US 1995-580166 19951228 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 24 OF 36 USPATFULL on STN
2000:18228 Hydrophobic u-PAR binding site.
Pessara, Ulrich, Penzberg, Germany, Federal Republic of
Weidle, Ulrich, Munchen, Germany, Federal Republic of
Konig, Bernhard, Berg, Germany, Federal Republic of
Kohnert, Ulrich, Habach, Germany, Federal Republic of
Bartke, Ilse, Bernried, Germany, Federal Republic of
Dan.o slashed. , Keld, Charlottenlund, Denmark
Ploug, Michael, Copenhagen, Denmark
Ellis, Vincent, Woodford Green, United Kingdom
Boehringer Mannheim GmbH, Mannheim, Germany, Federal Republic of (non-U.S. corporation)Cancerforskningsfonden af 1989, Copenhagen K, Denmark (non-U.S. corporation)
US 6025142 20000215
APPLICATION: US 1995-458585 19950602 (8)
PRIORITY: DK 1994-831 19940708
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 25 OF 36 USPATFULL on STN
1999:72710 Iterative method of at least five cycles for the refolding of proteins.
Th.o slashed.gersen, Christian, Mundelstrup, Denmark
Holtet, Thor Las, Aarhus V, Denmark

Bezerdt, Michael, Hillerup, Denmark
Denzyme APS, Aarhus C, Denmark (non-U.S. corporation)
US 5917018 19990629
APPLICATION: US 1995-469658 19950918 (8)
PRIORITY: DK 1993-130 19930204
DK 1993-139 19930205
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 26 OF 36 USPATFULL on STN
1999:43489 Affinity selection of ligands by mass spectroscopy.
Dollinger, Gavin D., San Francisco, CA, United States
Huebner, Verena D., Benicia, CA, United States
Kaur, Surinder, Lafayette, CA, United States
Chiron Corporation, Emeryville, CA, United States (U.S. corporation)
US 5891742 19990406
APPLICATION: US 1995-375979 19950119 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 27 OF 36 USPATFULL on STN
1999:43412 Vectors and methods for recombinant production of uPA-binding fragments of the human **urokinase**-type plasminogen receptor (uPAR).
Dan.o slashed. , Keld, Charlottenlund, Denmark
Blasi, Francesco, Charlottenlund, Denmark
Roldan, Ann Louring, Vallensb.ae butted.k, Denmark
Cubellis, Maria Vittoria, Napoli, Italy
Masucci, Maria Teresa, Napoli, Italy
Appella, Ettore, Chevy Chase, MD, United States
Schleuning, Wolf-Dieter, Berlin, Germany, Federal Republic of
Behrendt, Niels, Bagsv.ae butted.rd, Denmark
R.o slashed.nne, Ebbe, Copenhagen, Denmark
Kristensen, Peter, Copenhagen, Denmark
Pollanen, Jari, Espoo, Finland
Salonen, Eeva-Marjatta, Espoo, Finland
Stephens, Ross W., Helsinki, Finland
Tapiovaara, Hannele, Helsinki, Finland
Vaheri, Antti, Kauniainen, Finland
M.o slashed.ller, Lisbeth Birk, Bagsv.ae butted.rd, Denmark
Ellis, Vincent, Copenhagen, Denmark
Lund, Leif R.o slashed.ge, Copenhagen, Denmark
Ploug, Michael, Copenhagen, Denmark
Pyke, Charles, S.o slashed.borg, Denmark
Patthy, Laszlo, Budapest, Hungary
Cancerforskningsfondet af 1989, Copenhagen K, Denmark (non-U.S. corporation)
US 5891664 19990406
APPLICATION: US 1994-319052 19941006 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 28 OF 36 USPATFULL on STN
1998:154382 Terminal complement inhibitor fusion proteins.
Rother, Russell, Cheshire, CT, United States
Rollins, Scott, Monroe, CT, United States
Squinto, Stephen P., Bethany, CT, United States
Alexion Pharmaceuticals, Inc., New Haven, CT, United States (U.S. corporation)
US 5847082 19981208
APPLICATION: US 1995-482148 19950607 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 29 OF 36 USPATFULL on STN
1998:39670 Interative method of at least three cycles for the refolding of proteins.

Hansen, Hans Christian, Munkebjerup, Denmark
Holtet, Thor Lns, Aarhus V, Denmark
Etzerodt, Michael, Hinnerup, Denmark
Denzyme APS, Aarhus C, Denmark (non-U.S. corporation)
US 5739281 19980414
APPLICATION: US 1995-469486 19950606 (8)
PRIORITY: DK 1993-130 19930204
DK 1993-139 19930205
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 30 OF 36 USPATFULL on STN
97:106796 Modification of plasminogen activators.
Blasi, Francesco, Teglgardsvej 19A, DK-2920 Charlottenlund, Denmark
Stoppelli, Maria Patrizia, IIa Trav. L. Bianchi, 389, I-80131 Napoli, Italy
Mastronicola, Maria Rosaria, Via Nicolardi, 109, I-80131 Napoli, Italy
Welinder, Karen Gjersing, Amosebakken 14, DK-2830 Virum, Denmark
Correas, Isabel, C. Doctor Esquerdo 140-1, 7c, E-28007 Madrid, Spain
US 5688503 19971118
APPLICATION: US 1995-441358 19950515 (8)
PRIORITY: DK 1989-1822 19890414
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 31 OF 36 USPATFULL on STN
97:56536 Retroviral vector particles expressing complement inhibitor activity.
Mason, James M., Wallingford, CT, United States
Squinto, Stephen P., Bethany, CT, United States
Alexion Pharmaceuticals, Inc., New Haven, CT, United States (U.S. corporation)
US 5643770 19970701
APPLICATION: US 1994-278630 19940721 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 32 OF 36 USPATFULL on STN
97:38607 Chimeric complement inhibitor proteins.
Fodor, William L., New Haven, CT, United States
Rollins, Scott, Monroe, CT, United States
Squinto, Stephen P., Bethany, CT, United States
Alexion Pharmaceuticals, Inc., New Haven, CT, United States (U.S. corporation)
US 5627264 19970506
APPLICATION: US 1994-205508 19940303 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 33 OF 36 USPATFULL on STN
97:38553 Branched combinatorial libraries.
Valerio, Robert, Cranbourne South, Australia
Wang, Jian-Xin, Melbourne, Australia
Chiron Corporation, Emervylle, CA, United States (U.S. corporation)
US 5627210 19970506
APPLICATION: US 1995-385112 19950206 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 34 OF 36 USPATFULL on STN
97:36098 Nucleic acid encoding chimeric complement inhibitor proteins.
Fodor, William L., New Haven, CT, United States
Rollins, Scott, Monroe, CT, United States
Squinto, Stephen P., Bethany, CT, United States
Alexion Pharmaceuticals, Inc., New Haven, CT, United States (U.S. corporation)
US 5624837 19970429
APPLICATION: US 1995-458084 19950601 (8)

DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 35 OF 36 USPATFULL on STN
96:43770 **Urokinase**-type plasminogen activator receptor antibodies.
Dano, Keld, Charlottenlund, Denmark
Ronne, Ebbe, Copenhagen, Denmark
Behrendt, Niels, Bagsvaerd, Denmark
Ellis, Vincent, Copenhagen, Denmark
Hoyer-Hansen, Gunilla, Gentofte, Denmark
Pyke, Charles, Soborg, Denmark
Bruenner, Nils, Virum, Denmark
Cancerforskningsfondet af 1989, Copenhagen, Denmark (non-U.S. corporation)
US 5519120 19960521
APPLICATION: US 1993-85122 19930617 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L9 ANSWER 36 OF 36 USPATFULL on STN
95:43167 Modification of plasminogen activators.
Blasi, Francesco, Tlglg.ang.rdsvej 19A,, DK-2920 Charlottenlund, Denmark
Stoppelli, Maria P., IIa Trav. L. Bianchi, 389,, I-80131 Napoli, Italy
Mastronicola, Maria R., Via Nicolardi, 109,, I-80131 Napoli, Italy
Welinder, Karen G., Åmosebakken 14,, DK-2830 Virum, Denmark
Correas, Isabel, C. Doctor Esquerdo 140-1, 7c, E-28007 Madrid, Spain
US 5416006 19950516
WO 9012872 19901101
APPLICATION: US 1991-603675 19911218 (7)
WO 1990-DK96 19900411 19911218 PCT 371 date 19911218 PCT 102(e) date
PRIORITY: DK 1989-1822 19890414
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d l9,cbib,ab,clm,3,10,27,36

L9 ANSWER 3 OF 36 USPATFULL on STN
2004:2116 Human tissue **urokinase** type plasminogen activator production.
Hung, Paul Porwen, Bryn Mawr, PA, UNITED STATES
Wu, Bryan T. H., Taipei, TAIWAN, PROVINCE OF CHINA
Global Biotech, Inc. a Taiwan corporation (U.S. corporation)
US 2004002137 A1 20040101
APPLICATION: US 2003-401077 A1 20030327 (10)
PRIORITY: US 2002-371013P 20020409 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention features a nucleic acid expression vector that includes a bicistronic coding unit that comprises a first segment that encodes a human tissue **urokinase** plasminogen activator protein and a second segment that encodes an amplifiable dominant selectable marker (e.g., dihydrofolate reductase); and a promoter (e.g., a cytomegalovirus promoter) operably linked to the bicistronic coding unit.

CLM What is claimed is:

1. A nucleic acid expression vector comprising: a bicistronic coding unit that comprises a first segment that encodes a human tissue **urokinase** plasminogen activator protein and a second segment that encodes an amplifiable dominant selectable marker; and a promoter operably linked to the bicistronic coding unit.

2. The expression vector of claim 1, wherein the amplifiable dominant selectable marker is dihydrofolate reductase.

3. The expression vector of claim 2, wherein the human tissue **urokinase** plasminogen activator protein comprises SEQ ID NO:1.

4. The expression vector of claim 1, wherein the promoter is a

cytomegalovirus promoter.

5. The expression vector of claim 4, wherein the human tissue **urokinase** plasminogen activator protein comprises SEQ ID NO:1.

6. The expression vector of claim 1, wherein the human tissue **urokinase** plasminogen activator protein comprises SEQ ID NO:1.

7. The expression vector of claim 6, wherein the amplifiable dominant selectable marker is dihydrofolate reductase.

8. The expression vector of claim 6, wherein the promoter is a cytomegalovirus promoter.

9. A nucleic acid expression vector comprising: a bicistronic coding unit that comprises a first segment that encodes a human tissue **urokinase** plasminogen activator protein comprising SEQ ID NO:1 and a second segment that encodes dihydrofolate reductase; and a cytomegalovirus promoter operably linked to the bicistronic coding unit.

10. A mammalian CHO host cell comprising a nucleic acid which comprises: a bicistronic coding unit that comprises a first segment that encodes a human tissue **urokinase** plasminogen activator protein and a second segment that encodes an amplifiable dominant selectable marker; and a promoter operably linked to the bicistronic coding unit.

11. The mammalian host cell of claim 10, wherein the amplifiable dominant selectable marker is dihydrofolate reductase.

12. The mammalian host cell of claim 10, wherein the promoter is a cytomegalovirus promoter.

13. The mammalian host cell of claim 10, wherein the human tissue **urokinase** plasminogen activator protein comprises SEQ ID NO:1.

14. The mammalian host cell of claim 13, wherein the amplifiable dominant selectable marker is dihydrofolate reductase.

15. The mammalian host cell of claim 14, wherein the promoter is a cytomegalovirus promoter.

16. A method for producing a recombinant human tissue **urokinase** plasminogen activator protein, comprising: culturing a mammalian host cell of claim 10 in a medium under conditions promoting expression of the bicistronic coding unit and production of the human tissue **urokinase** plasminogen activator protein; and isolating the human tissue **urokinase** plasminogen activator protein from the cultured mammalian host cell or the medium.

17. The method of claim 16, wherein the amplifiable dominant selectable marker is dihydrofolate reductase.

18. The method of claim 16, wherein the promoter is a cytomegalovirus promoter.

19. The method of claim 16, wherein the human tissue **urokinase** plasminogen activator protein comprises SEQ ID NO: 1.

20. The method of claim 19, wherein the amplifiable dominant selectable marker is dihydrofolate reductase.

21. The method of claim 20, wherein the promoter is a cytomegalovirus promoter.

2003:112949 Human tissue **urokinase** type plasminogen activator formulation.
Hung, Paul Porwen, Bryn Mawr, PA, UNITED STATES
Chang, Kenneth S. S., Taipei, JAPAN
Wu, Bryan T. H., Taipei, JAPAN
Huang, Kuo-Kuei, Hsinchu, TAIWAN, PROVINCE OF CHINA
US 2003077682 A1 20030424
APPLICATION: US 2002-236881 A1 20020905 (10)
PRIORITY: US 2001-318173P 20010907 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB This invention relates to an anhydrous arginine-free formulation including human tissue **urokinase** type plasminogen activator, lysine, phosphoric acid, and a non-ionic detergent, wherein the human tissue **urokinase** type plasminogen activator, lysine, phosphoric acid, and non-ionic detergent are in quantities of 10-60 mg, 100-700 mg, 20-100 mg, and 0.2-5 mg, respectively; or in quantities of the same relative ratio.

CLM What is claimed is:

1. An anhydrous arginine-free formulation comprising: human tissue **urokinase** type plasminogen activator; lysine; phosphoric acid; and a non-ionic detergent; wherein the human tissue **urokinase** type plasminogen activator, lysine, phosphoric acid, and non-ionic detergent are in quantities of 10-60 mg, 100-700 mg, 20-100 mg, and 0.2-5 mg, respectively; or in quantities of the same relative ratio.

2. The formulation of claim 1, wherein the human tissue **urokinase** type plasminogen activator, lysine, phosphoric acid, and non-ionic detergent are in quantities of 15-60 mg, 150-500 mg, 30-80 mg, and 0.3-3 mg, respectively; or in quantities of the same relative ratio.

3. The formulation of claim 2, wherein the non-ionic detergent is polysorbate 20.

4. The formulation of claim 2, wherein the non-ionic detergent is polysorbate 80.

5. The formulation of claim 1, wherein the human tissue **urokinase** type plasminogen activator, lysine, phosphoric acid, and non-ionic detergent are in quantities of 30-40 mg, 200-350 mg, 40-60 mg, and 0.4-0.8 mg, respectively; or in quantities of the same relative ratio.

6. The formulation of claim 5, wherein the non-ionic detergent is polysorbate 20.

7. The formulation of claim 5, wherein the non-ionic detergent is polysorbate 80.

8. The formulation of claim 1, wherein the human tissue **urokinase** type plasminogen activator, lysine, phosphoric acid, and non-ionic detergent are in quantities of 35 mg, 200 mg, 50 mg, and 0.5 mg, respectively; or in quantities of the same relative ratio.

9. The formulation of claim 8, wherein the non-ionic detergent is polysorbate 20.

10. The formulation of claim 8, wherein the non-ionic detergent is polysorbate 80.

11. The formulation of claim 1, wherein the non-ionic detergent is polysorbate 20.

12. The formulation of claim 1, wherein the non-ionic detergent is polysorbate 80.

13. An anhydrous arginine-free formulation comprising 5-20% by weight human tissue **urokinase** type plasminogen activator and 55-85% by weight

lysine.

14. The formulation of claim 13, wherein the formulation comprises 8-16% by weight human tissue **urokinase** type plasminogen activator and 60-80% by weight lysine.

15. The formulation of claim 14, further comprising 15-20% by weight phosphoric acid.

16. The formulation of claim 15, further comprising 0.15-0.2% by weight a non-ionic detergent.

17. The formulation of claim 14, further comprising 0.15-0.2% by weight a non-ionic detergent.

18. The formulation of claim 13, wherein the formulation comprises 10-14% by weight human tissue **urokinase** type plasminogen activator and 65-75% by weight lysine.

19. The formulation of claim 18, further comprising 15-20% by weight phosphoric acid.

20. The formulation of claim 19, further comprising 0.15-0.2% by weight a non-ionic detergent.

21. The formulation of claim 18, further comprising 0.15-0.2% by weight a non-ionic detergent.

22. The formulation of claim 13, further comprising 15-20% by weight phosphoric acid.

23. The formulation of claim 22, further comprising 0.15-0.2% by weight a non-ionic detergent.

24. The formulation of claim 13, further comprising 0.15-0.2% by weight a non-ionic detergent.

L9 ANSWER 27 OF 36 USPATFULL on STN
1999:43412 Vectors and methods for recombinant production of uPA-binding fragments of the human **urokinase**-type plasminogen receptor (uPAR).
Dan.o slashed. , Keld, Charlottenlund, Denmark
Blasi, Francesco, Charlottenlund, Denmark
Roldan, Ann Louring, Vallensb.ae butted.k, Denmark
Cubellis, Maria Vittoria, Napoli, Italy
Masucci, Maria Teresa, Napoli, Italy
Appella, Ettore, Chevy Chase, MD, United States
Schleuning, Wolf-Dieter, Berlin, Germany, Federal Republic of
Behrendt, Niels, Bagsv.ae butted.rd, Denmark
R.o slashed.nne, Ebbe, Copenhagen, Denmark
Kristensen, Peter, Copenhagen, Denmark
Pollanen, Jari, Espoo, Finland
Salonen, Eeva-Marjatta, Espoo, Finland
Stephens, Ross W., Helsinki, Finland
Tapiovaara, Hannele, Helsinki, Finland
Vaheri, Antti, Kauniainen, Finland
M.o slashed.ller, Lisbeth Birk, Bagsv.ae butted.rd, Denmark
Ellis, Vincent, Copenhagen, Denmark
Lund, Leif R.o slashed.ge, Copenhagen, Denmark
Ploug, Michael, Copenhagen, Denmark
Pyke, Charles, S.o slashed.borg, Denmark
Patthy, Laszlo, Budapest, Hungary
Cancerforskningsfondet af 1989, Copenhagen K, Denmark (non-U.S. corporation)
US 5891664 19990406
APPLICATION: US 1994-319052 19941006 (8)

DOCUMENT TYPE: Utility, Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Activation of plasminogen to plasma is inhibited by preventing the binding of a receptor binding form of **urokinase**-type plasminogen activator to a **urokinase**-type plasminogen activator receptor in a mammal, thereby preventing the **urokinase**-type plasminogen activator from converting plasminogen into plasmin. DNA fragments which encode for soluble, active fragments of the **urokinase**-type plasminogen activator are provided.

CLM What is claimed is:

1. An expression vector which is capable of replicating in a host cell, said vector comprising (a) a coding sequence which encodes a polypeptide, soluble in aqueous solution, and having **urokinase** plasminogen activator (UPA) binding activity, said polypeptide comprising a UPA-binding domain having an amino acid sequence which (i) is identical to SEQ ID NO:3, (ii) is identical to the amino acid sequence of a UPA-binding 16 kDa glycosylated chymotryptic fragment of the mature uPA receptor protein having the amino acid sequence shown in sequence (A), or (iii) differs in amino acid sequence from (i) or (ii) above only by a single conservative amino acid substitution; (b) a stop codon immediately following said coding sequence; and (c) a promoter functional in said host cell and operably linked to said coding sequence; with the proviso that said vector does not comprise any sequence encoding a mature UPA receptor having the amino acid sequence shown in Sequence A or its natural precursor.

2. The vector of claim 1 wherein the polypeptide comprises a UPA-binding domain which is identical to SEQ ID NO:3, or which differs therefrom solely by a single conservative amino acid substitution of amino acids.

3. The vector of claim 1 wherein the polypeptide comprises a UPA-binding domain which is identical to SEQ ID NO:3.

4. The vector of claim 1 wherein the amino acid sequence of the polypeptide is identical to residues 1-281 of the mature human UPA-receptor having the sequence of Sequence A, or differs therefrom only by a carboxy terminal truncation and/or by one or more conservative substitutions of amino acids.

5. The expression vector of claim 4 in which the amino acid sequence of the polypeptide is identical to residues 1-281 of the mature human UPA-receptor having the sequence of Sequence A, or differs therefrom solely by one or more conservative substitutions.

6. The expression vector of claim 4 in which the amino acid sequence of the polypeptide is identical to residues 1-281 of the mature human UPA-receptor having the sequence of Sequence A, or to a fragment thereof which retains at least residues 1-92.

7. The expression vector of claim 4 in which the amino acid sequence of the polypeptide is identical to residues 1-281 of the mature human UPA-receptor having the sequence of Sequence A.

8. The expression vector of claim 4 in which the amino acid sequence of the polypeptide is identical to SEQ ID NO:3.

9. The vector of claim 1 where said polypeptide comprises an amino acid sequence corresponding to the amino acid sequence of a 16 kDa chymotryptic fragment of the UPA receptor which has UPA binding activity, or to a sequence differing from that of said fragment by a single conservative substitution.

10. The vector of claim 1 wherein the polypeptide comprises said uPA-binding peptide moiety and a plasminogen activator inhibitor moiety.

11. The vector of claim 1 wherein the polypeptide comprises said

uPA binding peptide moiety and a heterologous peptide moiety, where the heterologous peptide moiety increases expression of said polypeptide in said host cell or facilitates improves the purification or recovery of the polypeptide from said host cell.

12. A method of producing a polypeptide which is soluble in aqueous solution and has UPA binding activity which method comprises (a) providing a host cell transformed with a compatible expression vector according to claim 1; (b) expressing said coding sequence, whereby said polypeptide is produced in said cell, and (c) recovering said polypeptide.

13. The method of claim 12 wherein the polypeptide comprises a UPA-binding domain which is identical to SEQ ID NO:3, or which differs therefrom solely by a single conservative amino acid substitution of amino acids.

14. The method of claim 12, wherein the polypeptide comprises a UPA-binding domain which is identical to SEQ ID NO:3.

15. The method of claim 12, wherein the amino acid sequence of the polypeptide is identical to residues 1-281 of the mature human UPA-receptor having the sequence of Sequence A, or differs therefrom only by (A) a carboxy terminal truncation (B) one or more conservative substitutions of amino acids, or (C) both (A) and (B) above.

16. The method of claim 15, in which the amino acid sequence of the polypeptide is identical to residues 1-281 of the mature human UPA-receptor having the sequence of Sequence A, or differs therefrom only by one or more conservative substitutions.

17. The method of claim 15, in which the amino acid sequence of the polypeptide is identical to residues 1-281 of the mature human UPA-receptor having the sequence of Sequence A, or to a fragment thereof which retains at least residues 1-92.

18. The method of claim 15, in which the amino acid sequence of the polypeptide is identical to residues 1-281 of the mature human UPA-receptor having the sequence of Sequence A.

19. The method of claim 15, in which the amino acid sequence of the polypeptide is identical to SEQ ID NO:3.

20. The method of claim 12 where said polypeptide comprises an amino acid sequence corresponding to the amino acid sequence of a 16 kDa chymotryptic fragment of the UPA receptor which has UPA binding activity, or to a sequence differing from that of said fragment by a single conservative substitution.

21. The method of claim 12 wherein the polypeptide comprises said uPA-binding peptide moiety and a plasminogen activator inhibitor moiety.

22. The method of claim 12 wherein the polypeptide comprises said uPA-binding peptide moiety and a heterologous peptide moiety, where the heterologous peptide moiety increases expression of said polypeptide in said host cell or facilitates improves the purification or recovery of the polypeptide from said host cell.

L9 ANSWER 36 OF 36 USPATFULL on STN
95:43167 Modification of plasminogen activators.
Blasi, Francesco, T1glg.ang.rdsvej 19A,, DK-2920 Charlottenlund, Denmark
Stoppelli, Maria P., IIa Trav. L. Bianchi, 389,, I-80131 Napoli, Italy
Mastronicola, Maria R., Via Nicolardi, 109,, I-80131 Napoli, Italy
Welinder, Karen G., Åmosebakken 14,, DK-2830 Virum, Denmark
Correas, Isabel, C. Doctor Esquerdo 140-1, 7c, E-28007 Madrid, Spain

US 5418000 19950516
WO 9012872 19901101
APPLICATION: US 1991-603675 19911218 (7)
WO 1990-DK96 19900411 19911218 PCT 371 date 19911218 PCT 102(e) date
PRIORITY: DK 1989-1822 19890414
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Phosphorylated plasminogen activator, such as phosphorylated pro-**urokinase** (pro-u-PA), which is substantially free from unphosphorylated plasminogen activator, may be obtained by phosphorylating unphosphorylated plasminogen activator with a phosphorylating enzyme or by separating phosphorylated plasminogen activator from a mixture of phosphorylated plasminogen activator and unphosphorylated plasminogen activator. Phosphorylated pro-u-PA, which is substantially free from unphosphorylated pro-u-PA, is converted by plasmin into phosphorylated u-PA. The phosphorylated plasminogen activators such as phosphorylated pro-u-PA, u-PA and t-PA are useful as thrombolytic agents.

CLM What is claimed is:

1. A process for obtaining phosphorylated u-PA substantially free from unphosphorylated u-PA, which process comprises separating phosphorylated u-PA from a mixture of phosphorylated u-PA and unphosphorylated u-PA.

2. A process for obtaining phosphorylated pro-u-PA substantially free from unphosphorylated pro-u-PA, which process comprises separating phosphorylated pro-u-PA from a mixture of phosphorylated pro-u-PA and unphosphorylated pro-u-PA.

3. A process according to claim 1, which process comprises: (i) culturing a human cell line which produces u-PA; (ii) isolating the u-PA thus produced; and (iii) separating the phosphorylated u-PA from the unphosphorylated u-PA.

4. A process according to claim 2, which process comprises: (i) culturing a human cell line which produces pro-u-PA; (ii) isolating the pro-u-PA thus produced; and (iii) separating the phosphorylated pro-u-PA from the unphosphorylated pro-u-PA.

5. A process for the preparation of phosphorylated u-PA substantially free from unphosphorylated u-PA, which process comprises phosphorylating unphosphorylated u-PA with a phosphorylating enzyme.

6. A process for the preparation of phosphorylated pro-u-PA substantially free from unphosphorylated pro-u-PA, which process comprises phosphorylating unphosphorylated pro-u-PA with a phosphorylating enzyme.

7. A process according to claim 5, wherein a mixture of phosphorylated and unphosphorylated u-PA is treated with the phosphorylating enzyme.

8. A process according to claim 6, wherein a mixture of phosphorylated and unphosphorylated pro-u-PA is treated with the phosphorylating enzyme.

9. A process for the preparation of phosphorylated u-PA which is substantially free from unphosphorylated u-PA, which process comprises treating, with plasmin, phosphorylated pro-u-PA which is substantially free from unphosphorylated pro-u-PA.

10. A process for obtaining pro-u-PA wherein at least one of serine residues 138, 139 or 303 is phosphorylated and which is substantially free from unphosphorylated pro-u-PA, which process comprises separating said phosphorylated pro-u-PA from a mixture of phosphorylated pro-u-PA and unphosphorylated pro-u-PA.

11. A process for obtaining u-PA wherein at least one of serine residues

138, 139 or 303 is phosphorylated and which is substantially free from unphosphorylated u-PA, which process comprises separating said phosphorylated u-PA from a mixture of phosphorylated u-PA and unphosphorylated u-PA.

12. A process according to claim 10, which process comprises: (i) culturing a human cell line which produces pro-u-PA wherein at least one of serine residues 138, 139 or 303 is phosphorylated; (ii) isolating the pro-u-PA thus produced; and (iii) separating the phosphorylated pro-u-PA from the unphosphorylated pro-u-PA.

13. A process according to claim 11, which process comprises: (i) culturing a human cell line which produces u-PA wherein at least one of serine residues 138, 139 or 303 is phosphorylated; (ii) isolating the u-PA thus produced; and (iii) separating the phosphorylated u-PA from the unphosphorylated u-PA.

14. A process according to any of claims 1, 2, 10 or 11, wherein the separation is effected by Fe^{3+} -chelating chromatography.

15. A process for the preparation of phosphorylated pro-u-PA wherein least one of serine residues 138, 139 or 303 is phosphorylated which process comprises phosphorylating unphosphorylated pro-u-PA with a phosphorylating enzyme such that at least one of serine residues 138, 139 or 303 of the unphosphorylated pro-u-PA is phosphorylated.

16. A process for the preparation of phosphorylated u-PA wherein at least one of serine residues 138, 139 or 303 is phosphorylated which process comprises phosphorylating unphosphorylated u-PA with a phosphorylating enzyme such that at least one of serine residues 138, 139 or 303 of the unphosphorylated u-PA is phosphorylated.

17. A process for the preparation of phosphorylated u-PA wherein at least one of serine residues 138, 139 or 303 is phosphorylated and which u-PA is substantially free from unphosphorylated u-PA, which process comprises treating, with plasmin, phosphorylated pro-u-PA wherein at least one of serine residues 138, 139 or 303 is phosphorylated and which is substantially free from unphosphorylated pro-u-PA.

```
=> d his

     (FILE 'HOME' ENTERED AT 22:23:33 ON 10 MAR 2004)

     FILE 'USPATFULL' ENTERED AT 22:24:23 ON 10 MAR 2004
                E WADA MANABU/IN
L1            7 S E3
                E WADA NAOKO/IN
L2            2 S E3

     FILE 'MEDLINE' ENTERED AT 22:25:28 ON 10 MAR 2004
                E WADA M/AU
L3          875 S E3
L4            2 S L3 AND (UROKINASE)
                E WADA N/AU
L5            3 S E10

     FILE 'USPATFULL' ENTERED AT 22:28:52 ON 10 MAR 2004
L6         6244 S UROKINASE
L7           38 S L6 AND (HUMAN UROKINASE PLASMINOGEN ACTIVATOR)
L8            2 S L7 AND (HUMAN UROKINASE PLASMINOGEN ACTIVATOR/CLM)
L9           36 S L7 NOT L8

=> s l6 and (ATF or amino-terminal fragment)
          2115 ATF
```

```
        [illegible] AMINO
        625793 TERMINAL
         99459 FRAGMENT
           470 AMINO-TERMINAL FRAGMENT
                 (AMINO(W)TERMINAL(W)FRAGMENT)
L10        166 L6 AND (ATF OR AMINO-TERMINAL FRAGMENT)

=> s l10 and (ATF/clm or amino-terminal fragment/clm)
            92 ATF/CLM
        109541 AMINO/CLM
        192357 TERMINAL/CLM
         19596 FRAGMENT/CLM
            55 AMINO-TERMINAL FRAGMENT/CLM
                 ((AMINO(W)TERMINAL(W)FRAGMENT)/CLM)
L11         13 L10 AND (ATF/CLM OR AMINO-TERMINAL FRAGMENT/CLM)

=> d l11,cbib,1-13

L11  ANSWER 1 OF 13  USPATFULL on STN
2003:312240 Integrin ligand.
    Cress, Anne E., Tucson, AZ, UNITED STATES
    Edge, Albert, Newton, MA, UNITED STATES
    US 2003219837 A1 20031127
    APPLICATION: US 2003-382808  A1  20030306 (10)
    PRIORITY: US 2002-365370P  20020318 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11  ANSWER 2 OF 13  USPATFULL on STN
2003:285082 Adenovirus-mediated intratumoral delivery of an angiogenesis
    antagonist for the treatment of tumors.
    Li, Hong, Epinay sur Seine, FRANCE
    Lu, He, Epinay sur Seine, FRANCE
    Griscelli, Frank, Paris, FRANCE
    Opolon, Paule, Paris, FRANCE
    Soria, Claudine, Taverny, FRANCE
    Ragot, Thierry, Meudon, FRANCE
    Legrand, Yves, Paris, FRANCE
    Soria, Jeannette, Taverny, FRANCE
    Mabilat, Christelle, Corbeil Essonnes, FRANCE
    Perricaudet, Michel, Ecrosnes, FRANCE
    Yeh, Patrice, Gif sur Yvette, FRANCE
    Gencell SAS, Vitry sur Seine, FRANCE (non-U.S. corporation)
    US 6638502 B1 20031028
    WO 9849321  19981105
    APPLICATION: US 2000-403736  20000629 (9)
    WO 1998-EP2491 19980427
    PRIORITY: US 1997-44980P  19970428 (60)
    DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11  ANSWER 3 OF 13  USPATFULL on STN
2003:203309 Antithrombotic materials and methods.
    White, Mark L., Sonoma, CA, United States
    Ammons, William Steve, Pinole, CA, United States
    Xoma Corporation, Berkeley, CA, United States (U.S. corporation)
    US 6599881 B1 20030729
    APPLICATION: US 2000-610785  20000706 (9)
    DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11  ANSWER 4 OF 13  USPATFULL on STN
2003:180320 DTAT fusion toxin.
    Vallera, Daniel A., St. Louis Park, MN, UNITED STATES
    Hall, Walter A., Minneapolis, MN, UNITED STATES
    US 2003124147 A1 20030703
```

APPLICATION: US 2001-33577 A1 20011220 (10)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11 ANSWER 5 OF 13 USPATFULL on STN
2003:158911 Vectors for expressing multiple transgenes.
Diagana, Melissa, San Francisco, CA, UNITED STATES
Brockstedt, Dirk, Oakland, CA, UNITED STATES
US 2003108524 A1 20030612
APPLICATION: US 2002-273346 A1 20021018 (10)
PRIORITY: US 2001-329750P 20011018 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11 ANSWER 6 OF 13 USPATFULL on STN
2002:337939 Anti-HIV agents.
Wada, Manabu, Hyogo, JAPAN
Wada, Naoko, Hyogo, JAPAN
JCR Pharmaceuticals Co., Ltd., Hyogo, JAPAN, 659-0021 (non-U.S. corporation)
US 2002193304 A1 20021219
APPLICATION: US 2002-76421 A1 20020219 (10)
PRIORITY: JP 2001-42655 20010220
JP 2001-184284 20010619
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11 ANSWER 7 OF 13 USPATFULL on STN
2002:308339 Modulation of pericyte proliferation.
King, George L., Boston, MA, UNITED STATES
Abrahamson, Susan, Berkeley, CA, UNITED STATES
Pugsley, Michael, Pleasant Hill, CA, UNITED STATES
US 2002173464 A1 20021121
APPLICATION: US 2001-6557 A1 20011203 (10)
PRIORITY: US 2000-250542P 20001201 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11 ANSWER 8 OF 13 USPATFULL on STN
2002:242784 Compositions and methods for modulating muscle cell and tissue contractility.
Cines, Douglas B., Wynnewood, PA, UNITED STATES
Higazi, Abd Al-Roof, Jerusalem, ISRAEL
The Trustees of the University of Pennsylvania (U.S. corporation)
US 2002131964 A1 20020919
APPLICATION: US 2001-880503 A1 20010613 (9)
PRIORITY: US 2000-212874P 20000620 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11 ANSWER 9 OF 13 USPATFULL on STN
2001:93478 **Urokinase**-type plasminogen activator receptor.
Dan.o slashed. , Keld, Charlottenlund, Denmark
Blasi, Francesco, Charlottenlund, Denmark
Roldan, Ann Louring, Vallensb.ae butted.k, Denmark
Cubellis, Maria Vittoria, Naples, Italy
Masucci, Maria Teresa, Naples, Italy
Appella, Ettore, Chevy Chase, MD, United States
Schleuning, W.D., Berlin, Germany, Federal Republic of
Behrendt, Niels, Bagsv.ae butted.rd, Denmark
R.o slashed.nne, Ebbe, Copenhagen, Denmark
Kristensen, Peter, Copenhagen, Denmark
Pollanen, Jari, Espoo, Finland
Salonen, Eeva-Marjatta, Espoo, Finland
Stephens, Ross W., Vantaa, Finland
Tapiovaara, Hannele, Helsinki, Finland

Vahell, Antti, Kauhajoki, Finland
M.o slashed.ller, Lisbeth Birk, Bagsv.ae butted.rd, Denmark
Ellis, Vincent, Copenhagen, Denmark
Lund, Leif R.o slashed.ge, Copenhagen, Denmark
Ploug, Michael, Copenhagen, Denmark
Pyke, Charles, S.o slashed.borg, Denmark
Patthy, Laszlo, Budapest, Hungary
Cancerforskningsfondet af 1989, Denmark (non-U.S. corporation)
US 6248712 B1 20010619
APPLICATION: US 1995-442108 19950516 (8)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11 ANSWER 10 OF 13 USPATFULL on STN
2000:18228 Hydrophobic u-PAR binding site.
Pessara, Ulrich, Penzberg, Germany, Federal Republic of
Weidle, Ulrich, Munchen, Germany, Federal Republic of
Konig, Bernhard, Berg, Germany, Federal Republic of
Kohnert, Ulrich, Habach, Germany, Federal Republic of
Bartke, Ilse, Bernried, Germany, Federal Republic of
Dan.o slashed. , Keld, Charlottenlund, Denmark
Ploug, Michael, Copenhagen, Denmark
Ellis, Vincent, Woodford Green, United Kingdom
Boehringer Mannheim GmbH, Mannheim, Germany, Federal Republic of (non-U.S. corporation)Cancerforskningsfonden af 1989, Copenhagen K, Denmark (non-U.S. corporation)
US 6025142 20000215
APPLICATION: US 1995-458585 19950602 (8)
PRIORITY: DK 1994-831 19940708
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11 ANSWER 11 OF 13 USPATFULL on STN
1999:92646 Antithrombotic materials and methods.
White, Mark L., Sonoma, CA, United States
Ammons, William Steve, Pinole, CA, United States
Xoma Corporation, Berkeley, CA, United States (U.S. corporation)
US 5935930 19990810
APPLICATION: US 1998-63465 19980420 (9)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11 ANSWER 12 OF 13 USPATFULL on STN
1999:72457 Promoter for VEGF receptor.
Williams, Lewis T., Tiburon, CA, United States
Morishita, Kaoru, Tokyo, Japan
The Regents of the University of California, Oakland, CA, United States (U.S. corporation)
US 5916763 19990629
APPLICATION: US 1995-556424 19951109 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L11 ANSWER 13 OF 13 USPATFULL on STN
1998:42348 Antithrombotic materials and methods.
White, Mark L., Sonoma, CA, United States
Ammons, William Steve, Pinole, CA, United States
XOMA Corporation, Berkeley, CA, United States (U.S. corporation)
US 5741779 19980421
APPLICATION: US 1996-644290 19960510 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d l11

L11 ANSWER 1 OF 13 USPATFULL on STN
Full Text
AN 2003:312240 USPATFULL
TI Integrin ligand
IN Cress, Anne E., Tucson, AZ, UNITED STATES
Edge, Albert, Newton, MA, UNITED STATES
PI US 2003219837 A1 20031127
AI US 2003-382808 A1 20030306 (10)
PRAI US 2002-365370P 20020318 (60)
DT Utility
FS APPLICATION
LN.CNT 3845
INCL INCLM: 435/007.200
INCLS: 435/005.000
NCL NCLM: 435/007.200
NCLS: 435/005.000
IC [7]
ICM: C12Q001-70
ICS: G01N033-53; G01N033-567
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> d l11,cbib,ab,clm,13

L11 ANSWER 13 OF 13 USPATFULL on STN
1998:42348 Antithrombotic materials and methods.
White, Mark L., Sonoma, CA, United States
Ammons, William Steve, Pinole, CA, United States
XOMA Corporation, Berkeley, CA, United States (U.S. corporation)
US 5741779 19980421
APPLICATION: US 1996-644290 19960510 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Antithrombotic materials and methods are provided for the treatment of thrombotic disorders, in which therapeutically effective amounts of BPI protein products are administered.
CLM What is claimed is:
1. A method for slowing clot formation in blood comprising administering to a subject a BPI protein product in an amount effective to delay or prevent clot formation in the blood.

2. A method for enhancing clot dissolution in blood comprising administering to a subject a BPI protein product in an amount effective to enhance clot dissolution in the blood.

3. A method of slowing clot formation in blood comprising contacting the blood with an amount of BPI protein product effective to delay or prevent clot formation in the blood.

4. A method for enhancing clot dissolution in blood comprising contacting the blood with an amount of BPI protein product effective to dissolve or lyse the clot.

5. The method of any one of claims 1, 2, 3 or 4 wherein the BPI protein product is an **amino-terminal fragment** of BPI protein having a molecular weight of about 21 kD to 25 kD.

6. The method of any one of claims 1, 2, 3 or 4 wherein the BPI protein product is $rBPI_{23}$ or a dimeric form thereof.

7. The method of any one of claims 1, 2, 3 or 4 wherein the BPI protein product is $rBPI_{21}$.

=> d l11,cbib,ab,clm,kwic,13

L11 ANSWER 13 OF 13 USPATFULL on STN
1998:42348 Antithrombotic materials and methods.
White, Mark L., Sonoma, CA, United States
Ammons, William Steve, Pinole, CA, United States
XOMA Corporation, Berkeley, CA, United States (U.S. corporation)
US 5741779 19980421
APPLICATION: US 1996-644290 19960510 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Antithrombotic materials and methods are provided for the treatment of thrombotic disorders, in which therapeutically effective amounts of BPI protein products are administered.

CLM What is claimed is:

1. A method for slowing clot formation in blood comprising administering to a subject a BPI protein product in an amount effective to delay or prevent clot formation in the blood.

2. A method for enhancing clot dissolution in blood comprising administering to a subject a BPI protein product in an amount effective to enhance clot dissolution in the blood.

3. A method of slowing clot formation in blood comprising contacting the blood with an amount of BPI protein product effective to delay or prevent clot formation in the blood.

4. A method for enhancing clot dissolution in blood comprising contacting the blood with an amount of BPI protein product effective to dissolve or lyse the clot.

5. The method of any one of claims 1, 2, 3 or 4 wherein the BPI protein product is an **amino-terminal fragment** of BPI protein having a molecular weight of about 21 kD to 25 kD.

6. The method of any one of claims 1, 2, 3 or 4 wherein the BPI protein product is $rBPI_{23}$ or a dimeric form thereof.

7. The method of any one of claims 1, 2, 3 or 4 wherein the BPI protein product is $rBPI_{21}$.

SUMM The thrombolytic agents include tPA, streptokinase, **urokinase** prourokinase, anisolylated plasminogen streptokinase activation complex (APSAC), and animal salivary gland plasminogen activators, all of which act by accelerating fibrinolysis.. . .

SUMM . . . the treatment of thrombotic disorder by concurrent administration of a BPI protein product with a thrombolytic agent, including tPA, streptokinase, **urokinase**, prourokinase, APSAC, animal salivary gland plasminogen activators, other plasminogen activators, and derivatives of such plasminogen activators. According to this aspect. . .

SUMM . . . Thrombolytic agents are agents with the pharmacological effect of enhancing clot dissolution, and include plasminogen activators such as t-PA, streptokinase, **urokinase**, proutokinase, APSAC, animal salivary gland plasminogen activators and derivatives thereof.

SUMM **Urokinase** is indicated for lysis of acute pulmonary emboli and coronary artery emboli, and is also used to restore patency to. . . a period of 10 minutes followed by a continuous infusion of 2,000 units/lb/hr for 12 hours. The total dose of **urokinase** given will range from 2.25 million to 6.25 million units, depending on the weight of the patient. When it is used to clear intravenous cannulae or catheters, **urokinase** is given as a single injection of 5,000 units in a volume of 1 mL.

DETD Clot formation was evaluated when $rBPI_{21}$ and a plasminogen activator (tPA, **urokinase** or streptokinase) were added to PPP prior to calcium addition. The following reagents were added to each well of a.

. . 20 µL of $rBPI_{21}$ (1000, 250, 50, 10 or 2 µg/mL): (4) 20 µL of PA (tPA, 1000 ng/mL; for **urokinase**, 100 and 1000 ng/mL: for streptokinase, 100 and 1000 ng/mL); and (5) 50 µL of 20 mM $CaCl_2$ in TBS.. . .

DETD . . . over the 2 hour kinetic plate reader analysis), and at 25 µg/mL, it substantially prevented clotting under conditions where tPA, **urokinase** or streptokinase was present in the pre-clot mixture. Effects on decreasing clot formation were observed with 5 µg/mL $rBPI_{21}$. At 1 and 0.2 µg/mL, $rBPI_{21}$ clot formation time was comparable to the control. At the concentrations of **urokinase** tested (10 and 100 ng/mL), clot dissolution did not occur following clot formation, as it did with 100 ng/mL tPA. . .

. . . 5. The method of any one of claims 1, 2, 3 or 4 wherein the BPI protein product is an **amino-terminal fragment** of BPI protein having a molecular weight of about 21 kD to 25 kD.

```
=> file medline
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                         ENTRY    SESSION
FULL ESTIMATED COST                                      86.00      93.25

FILE 'MEDLINE' ENTERED AT 22:38:16 ON 10 MAR 2004

 FILE LAST UPDATED: 10 MAR 2004 (20040310/UP).  FILE COVERS 1953 TO DATE.

 On February 29, 2004, the 2004 MeSH terms were loaded.  See HELP RLOAD
 for details.

 MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the
 MeSH 2004 vocabulary.  See http://www.nlm.nih.gov/mesh/ and
 http://www.nlm.nih.gov/pubs/techbull/nd03/nd03_mesh.html for a
 description of changes.

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.

=> s (urokinase or human urokinase plasminogen activator)
          9092 UROKINASE
       8435981 HUMAN
          9092 UROKINASE
         31581 PLASMINOGEN
         57297 ACTIVATOR
            26 HUMAN UROKINASE PLASMINOGEN ACTIVATOR
                 (HUMAN(W)UROKINASE(W)PLASMINOGEN(W)ACTIVATOR)
L12        9092 (UROKINASE OR HUMAN UROKINASE PLASMINOGEN ACTIVATOR)

=> s l12 and (human urokinase plasminogen activator)
       8435981 HUMAN
          9092 UROKINASE
         31581 PLASMINOGEN
         57297 ACTIVATOR
            26 HUMAN UROKINASE PLASMINOGEN ACTIVATOR
                 (HUMAN(W)UROKINASE(W)PLASMINOGEN(W)ACTIVATOR)
L13          26 L12 AND (HUMAN UROKINASE PLASMINOGEN ACTIVATOR)

=> d l13,cbib,1-26

L13  ANSWER 1 OF 26     MEDLINE on STN
2004061937.    PubMed ID: 14741775.   Improved effects of viral gene delivery
     of human uPA plus biliodigestive anastomosis induce recovery from
     experimental biliary cirrhosis. Miranda-Diaz Alejandra; Rincon Ana Rosa;
     Salgado Silvia; Vera-Cruz Jose; Galvez Javier; Islas Ma Cristina; Berumen
     Jaime; Aguilar-Cordova Estuardo; Armendariz-Borunda Juan. (Institute of
```

Molecular Biology in Medicine and Gene Therapy, CUCS, University of Guadalajara, Apartado Postal 2-123, 44281, Guadalajara, Jalisco, Mexico.) Molecular therapy : journal of the American Society of Gene Therapy, (2004 Jan) 9 (1) 30-7. Journal code: 100890581. ISSN: 1525-0016. Pub. country: United States. Language: English.

L13 ANSWER 2 OF 26 MEDLINE on STN
2001270034. PubMed ID: 11085980. Regulation of **urokinase/urokinase** receptor interaction by heparin-like glycosaminoglycans. Pucci M; Fibbi G; Magnelli L; Del Rosso M. (Department of Experimental Pathology and Oncology of Florence University, Viale G. B. Morgagni 50, 50134 Florence, Italy.) Journal of biological chemistry, (2001 Feb 16) 276 (7) 4756-65. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

L13 ANSWER 3 OF 26 MEDLINE on STN
2000237098. PubMed ID: 10772921. Regulation of **urokinase** receptor transcription by Ras- and Rho-family GTPases. Muller S M; Okan E; Jones P. (School of Biomedical Sciences, Queen's Medical Centre, Nottingham, NG7 2UH, United Kingdom.) Biochemical and biophysical research communications, (2000 Apr 21) 270 (3) 892-8. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

L13 ANSWER 4 OF 26 MEDLINE on STN
97449126. PubMed ID: 9305622. Lysine 156 promotes the anomalous proenzyme activity of tPA: X-ray crystal structure of single-chain human tPA. Renatus M; Engh R A; Stubbs M T; Huber R; Fischer S; Kohnert U; Bode W. (Max-Planck-Institute of Biochemistry, Department of Structural Research, Martinsried, Germany.. renatus@biochem.mpg.de) . EMBO journal, (1997 Aug 15) 16 (16) 4797-805. Journal code: 8208664. ISSN: 0261-4189. Pub. country: ENGLAND: United Kingdom. Language: English.

L13 ANSWER 5 OF 26 MEDLINE on STN
96261999. PubMed ID: 8656020. Binding of human **urokinase** type plasminogen activator and plasminogen to Borrelia species. Klempner M S; Noring R; Epstein M P; McCloud B; Rogers R A. (Department of Medicine, New England Medical Center, Tufts University School of Medicine, Boston, Massachusetts 02111, USA.) Journal of infectious diseases, (1996 Jul) 174 (1) 97-104. Journal code: 0413675. ISSN: 0022-1899. Pub. country: United States. Language: English.

L13 ANSWER 6 OF 26 MEDLINE on STN
96140764. PubMed ID: 8557253. A highly polymorphic CA/GT repeat in intron 3 of the human **urokinase** receptor gene (PLAUR). Kohonen-Corish M R; Wang Y; Doe W F. (Division of Clinical Sciences, John Curtin School of Medical Research, Australian National University, Canberra, Australia.) Human genetics, (1996 Jan) 97 (1) 124-5. Journal code: 7613873. ISSN: 0340-6717. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

L13 ANSWER 7 OF 26 MEDLINE on STN
96050896. PubMed ID: 8556526. Role of cytoskeletal elements in expression of monocyte **urokinase** plasminogen activator receptor, activation-associated antigen Mo3. Washington R; Dore-Duffy P. (Department of Neurology, Wayne State University School of Medicine, Detroit, Michigan 48201, USA.) Clinical and diagnostic laboratory immunology, (1994 Nov) 1 (6) 714-21. Journal code: 9421292. ISSN: 1071-412X. Pub. country: United States. Language: English.

L13 ANSWER 8 OF 26 MEDLINE on STN
96016640. PubMed ID: 8532668. Yeast expression and phagemid display of the **human urokinase plasminogen activator** epidermal growth factor-like domain. Stratton-Thomas J R; Min H Y; Kaufman S E; Chiu C Y; Mullenbach G T; Rosenberg S. (Chiron Corporation, Emeryville, CA 94608, USA.) Protein engineering, (1995 May) 8 (5) 463-70. Journal code: 8801484. ISSN: 0269-2139. Pub. country: ENGLAND: United Kingdom. Language: English.

L13 ANSWER 9 OF 26 MEDLINE on STN
95217327. PubMed ID: 7702750. Regulation of the **urokinase** gene by the retinoblastoma protein. Novak U; Paradiso L; Hamilton J A. (University of Melbourne, Department of Medicine, Royal Melbourne Hospital, Parkville, Victoria, Australia.) DNA and cell biology, (1994 Nov) 13 (11) 1063-9. Journal code: 9004522. ISSN: 1044-5498. Pub. country: United States. Language: English.

L13 ANSWER 10 OF 26 MEDLINE on STN
95035933. PubMed ID: 7948815. Chromosomal localization of the **human urokinase plasminogen activator** receptor and plasminogen activator inhibitor type-2 genes: implications in colorectal cancer. Webb G; Baker M S; Nicholl J; Wang Y; Woodrow G; Kruithof E; Doe W F. (Division of Clinical Sciences, John Curtin School of Medical Research, Australian National University, Canberra.) Journal of gastroenterology and hepatology, (1994 Jul-Aug) 9 (4) 340-3. Journal code: 8607909. ISSN: 0815-9319. Pub. country: Australia. Language: English.

L13 ANSWER 11 OF 26 MEDLINE on STN
94350977. PubMed ID: 8071349. Differential DNA sequence specificity and regulation of HIV-1 enhancer activity by cRel-RelA transcription factor. Hansen S K; Guerrini L; Blasi F. (Department of Genetics and Microbiol Biology, University of Milano, Italy.) Journal of biological chemistry, (1994 Sep 2) 269 (35) 22230-7. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

L13 ANSWER 12 OF 26 MEDLINE on STN
94043730. PubMed ID: 8227331. A ligand-free, soluble **urokinase** receptor is present in the ascitic fluid from patients with ovarian cancer. Pedersen N; Schmitt M; Ronne E; Nicoletti M I; Hoyer-Hansen G; Conese M; Giavazzi R; Dano K; Kuhn W; Janicke F; +. (Institute of Microbiology, University of Copenhagen, Denmark.) Journal of clinical investigation, (1993 Nov) 92 (5) 2160-7. Journal code: 7802877. ISSN: 0021-9738. Pub. country: United States. Language: English.

L13 ANSWER 13 OF 26 MEDLINE on STN
93314820. PubMed ID: 8392005. An alternatively spliced variant of mRNA for the human receptor for **urokinase** plasminogen activator. Pyke C; Eriksen J; Solberg H; Nielsen B S; Kristensen P; Lund L R; Dano K. (Finsen Laboratory, Rigshospitalet, Copenhagen, Denmark.) FEBS letters, (1993 Jul 12) 326 (1-3) 69-74. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

L13 ANSWER 14 OF 26 MEDLINE on STN
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L13 ANSWER 16 OF 26 MEDLINE on STN
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Research, (1991 May 11) 19 (9) 2305 0. Journal code: 0411011. ISSN: 0305-1048. Pub. country: ENGLAND: United Kingdom. Language: English.

L13 ANSWER 17 OF 26 MEDLINE on STN
91224949. PubMed ID: 1851152. A soluble, ligand binding mutant of the **human urokinase plasminogen activator** receptor. Masucci M T; Pedersen N; Blasi F. (Institute of Microbiology, University of Copenhagen, Denmark.) Journal of biological chemistry, (1991 May 15) 266 (14) 8655-8. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

L13 ANSWER 18 OF 26 MEDLINE on STN
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L13 ANSWER 20 OF 26 MEDLINE on STN
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L13 ANSWER 21 OF 26 MEDLINE on STN
90151622. PubMed ID: 1689240. Cloning and expression of the receptor for **human urokinase plasminogen activator**, a central molecule in cell surface, plasmin dependent proteolysis. Roldan A L; Cubellis M V; Masucci M T; Behrendt N; Lund L R; Dano K; Appella E; Blasi F. (University Institute of Microbiology, Copenhagen, Denmark.) EMBO journal, (1990 Feb) 9 (2) 467-74. Journal code: 8208664. ISSN: 0261-4189. Pub. country: ENGLAND: United Kingdom. Language: English.

L13 ANSWER 22 OF 26 MEDLINE on STN
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L13 ANSWER 23 OF 26 MEDLINE on STN
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L13 ANSWER 24 OF 26 MEDLINE on STN
87057228. PubMed ID: 3023326. Binding of single-chain prourokinase to the **urokinase** receptor of human U937 cells. Cubellis M V; Nolli M L; Cassani G; Blasi F. Journal of biological chemistry, (1986 Dec 5) 261 (34) 15819-22. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

L13 ANSWER 25 OF 26 MEDLINE on STN
85270442. PubMed ID: 2991901. Differentiation-enhanced binding of the amino-terminal fragment of **human urokinase plasminogen activator** to a specific receptor on U937 monocytes. Stoppelli M P; Corti A; Soffientini A; Cassani G; Blasi F; Assoian R K. Proceedings of the National Academy of Sciences of the United States of America, (1985 Aug) 82 (15) 4939-43. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

L13 ANSWER 26 OF 26 MEDLINE on STN
85215647. PubMed ID: 2987867. The **human urokinase-plasminogen activator** gene and its promoter. Riccio A; Grimaldi G; Verde P; Sebastio G; Boast S; Blasi F. Nucleic acids research, (1985 Apr 25) 13 (8) 2759-71. Journal code: 0411011. ISSN: 0305-1048. Pub. country: ENGLAND: United Kingdom. Language: English.

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L13 ANSWER 1 OF 26 MEDLINE on STN
2004061937. PubMed ID: 14741775. Improved effects of viral gene delivery of human uPA plus biliodigestive anastomosis induce recovery from experimental biliary cirrhosis. Miranda-Diaz Alejandra; Rincon Ana Rosa; Salgado Silvia; Vera-Cruz Jose; Galvez Javier; Islas Ma Cristina; Berumen Jaime; Aguilar-Cordova Estuardo; Armendariz-Borunda Juan. (Institute of Molecular Biology in Medicine and Gene Therapy, CUCS, University of Guadalajara, Apartado Postal 2-123, 44281, Guadalajara, Jalisco, Mexico.) Molecular therapy : journal of the American Society of Gene Therapy, (2004 Jan) 9 (1) 30-7. Journal code: 100890581. ISSN: 1525-0016. Pub. country: United States. Language: English.

AB Gene therapy may represent a new avenue for the development of multimodal treatment for diverse forms of cirrhosis. This study explores the potential benefits of combining adenovirus-mediated **human urokinase-plasminogen activator** (AdHuPA) gene delivery and biliodigestive anastomosis to enhance the therapeutic efficacy of each treatment alone for cholestatic disorders resulting in secondary biliary cirrhosis. In an experimental model of secondary biliary cirrhosis, application of 6 x 10(11) vp/kg AdHuPA adenovirus vector resulted in 25.8% liver fibrosis reduction and some improvement in liver histology. The relief of bile cholestasis by a surgical procedure (biliodigestive anastomosis) combined with AdHuPA hepatic gene delivery rendered a synergistic effect, with a substantial 56.9 to 42.9% fibrosis decrease. AdHuPA transduction resulted in clear-cut expression of human uPA protein detected by immunohistochemistry and induction of up-regulation in the expression of metalloproteinases MMP-3, MMP-9, and MMP-2. Importantly, functional hepatic tests, specifically direct bilirubin, were improved. Also, hepatic cell regeneration, rearrangement of hepatic architecture, ascites, and gastric varices improved in cirrhotic rats treated with AdHuPA but not in counterpart AdGFP cirrhotic animals. We believe this might represent a novel therapeutic strategy for human cholestatic diseases.

L13 ANSWER 2 OF 26 MEDLINE on STN
2001270034. PubMed ID: 11085980. Regulation of **urokinase/urokinase** receptor interaction by heparin-like glycosaminoglycans. Pucci M; Fibbi G; Magnelli L; Del Rosso M. (Department of Experimental Pathology and Oncology of Florence University, Viale G. B. Morgagni 50, 50134 Florence, Italy.) Journal of biological chemistry, (2001 Feb 16) 276 (7) 4756-65. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB We show here that the interaction between the **urokinase**-type plasminogen activator and its receptor, which plays a critical role in cell invasion, is regulated by heparan sulfate present on the cell surface and in the extracellular matrix. Heparan sulfate oligomers showing a composition close to the dimeric repeats of heparin (glucosamine-NSO(3)(6-OSO(3))-

iduronic acid(2 OSO(3)) n = 3 and n > 3, where iduronic acid may alternate with glucuronic acid, exhibit affinity for **urokinase** plasminogen activator and confer specificity on **urokinase/urokinase** receptor interaction. Cell surface clearance of heparan sulfate reduces the affinity of such interaction with a parallel decrease of specific **urokinase** binding in the presence of an unaltered expression of receptor. Transfection of **human urokinase plasminogen activator** receptor in normal Chinese hamster ovary fibroblasts and in Chinese hamster ovary cells defective for the synthesis of sulfated glycosaminoglycans results in specific **urokinase**/receptor interaction only in nondefective cells. Heparan sulfate/**urokinase** and receptor/**urokinase** interactions exhibit similar K(d) values. We concluded that heparan sulfate functions as an adaptor molecule that confers specificity on **urokinase**/receptor binding.

L13 ANSWER 3 OF 26 MEDLINE on STN
2000237098. PubMed ID: 10772921. Regulation of **urokinase** receptor transcription by Ras- and Rho-family GTPases. Muller S M; Okan E; Jones P. (School of Biomedical Sciences, Queen's Medical Centre, Nottingham, NG7 2UH, United Kingdom.) Biochemical and biophysical research communications, (2000 Apr 21) 270 (3) 892-8. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.
AB How cell adhesion is coordinated with extracellular proteolysis is a key question in understanding cell migration. Potentially, the small GTP-binding proteins that affect actin organisation and signal transduction may also regulate the expression of genes associated with extracellular proteolysis. We investigated the ability of Ras, Rac-1, Cdc42Hs, and RhoA to regulate transcription from the1.55-kb promoter region of the **human urokinase plasminogen activator** receptor (uPAR) gene. Constitutively active V12 H-Ras and Rho-A stimulated uPAR transcription while Cdc42Hs and Rac-1 did not. The use of Ras effector-loop mutants indicated that signalling via multiple Ras-effectors is necessary for the maximum activation of transcription. Copyright 2000 Academic Press.

L13 ANSWER 4 OF 26 MEDLINE on STN
97449126. PubMed ID: 9305622. Lysine 156 promotes the anomalous proenzyme activity of tPA: X-ray crystal structure of single-chain human tPA. Renatus M; Engh R A; Stubbs M T; Huber R; Fischer S; Kohnert U; Bode W. (Max-Planck-Institute of Biochemistry, Department of Structural Research, Martinsried, Germany.. renatus@biochem.mpg.de) . EMBO journal, (1997 Aug 15) 16 (16) 4797-805. Journal code: 8208664. ISSN: 0261-4189. Pub. country: ENGLAND: United Kingdom. Language: English.
AB Tissue type plasminogen activator (tPA) is the physiological initiator of fibrinolysis, activating plasminogen via highly specific proteolysis; plasmin then degrades fibrin with relatively broad specificity. Unlike other chymotrypsin family serine proteinases, tPA is proteolytically active in a single-chain form. This form is also preferred for therapeutic administration of tPA in cases of acute myocardial infarction. The proteolytic cleavage which activates most other chymotrypsin family serine proteinases increases the catalytic efficiency of tPA only 5- to 10-fold. The X-ray crystal structure of the catalytic domain of recombinant human single-chain tPA shows that Lys156 forms a salt bridge with Asp194, promoting an active conformation in the single-chain form. Comparisons with the structures of other serine proteinases that also possess Lys156, such as trypsin, factor Xa and **human urokinase plasminogen activator** (uPA), identify a set of secondary interactions which are required for Lys156 to fulfil this activating role. These findings help explain the anomalous single-chain activity of tPA and may suggest strategies for design of new therapeutic plasminogen activators.

L13 ANSWER 5 OF 26 MEDLINE on STN
96261999. PubMed ID: 8656020. Binding of human **urokinase** type plasminogen activator and plasminogen to Borrelia species. Klempner M S; Noring R; Epstein M P; McCloud B; Rogers R A. (Department of Medicine, New England Medical Center, Tufts University School of Medicine, Boston,

Massachusetts 02111, USA.) Journal of infectious diseases, (1996 Jul) 174 (1) 97-104. Journal code: 0413675. ISSN: 0022-1899. Pub. country: United States. Language: English.

AB Borrelia burgdorferi binds **human urokinase plasminogen activator** (uPA), which cleaves plasminogen (Pgn) to plasmin. The ability of other Borrelia species to bind uPA, Pgn, or both was investigated. Borrelia coriacae, Borrelia garinii, Borrelia parkeri, Borrelia anserina, and Borrelia turicatae were compared with infectious and noninfectious B. burgdorferi isolates. All Borrelia species lacked endogenous proteases capable of digesting casein, but all species bound human uPA and Pgn, generating Pgn-dependent proteolytic activity. There were no significant differences in the amount of plasmin, Pgn, or uPA bound per spirochete of the different species. On unfixed borreliae, fluorochrome-conjugated uPA bound to all species. Early binding was at the terminus of B. burgdorferi, whereas diffuse binding was observed on B. coriacae, B. garinii, B. parkeri, and B. turicatae. These studies demonstrate that binding of human uPA and Pgn to borreliae occurs on multiple species with apparent differences in surface distribution.

L13 ANSWER 6 OF 26 MEDLINE on STN

96140764. PubMed ID: 8557253. A highly polymorphic CA/GT repeat in intron 3 of the human **urokinase** receptor gene (PLAUR). Kohonen-Corish M R; Wang Y; Doe W F. (Division of Clinical Sciences, John Curtin School of Medical Research, Australian National University, Canberra, Australia.) Human genetics, (1996 Jan) 97 (1) 124-5. Journal code: 7613873. ISSN: 0340-6717. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB We describe the first highly polymorphic microsatellite marker for the **human urokinase plasminogen activator** receptor gene (PLAUR). The **urokinase** receptor (uPAR) has a central role in cancer invasion and metastasis, which may enable the development of new anti-metastatic therapies. Analysis of the marker genotypes in colorectal cancer cell lines revealed three alleles that were not detected in a series of healthy control individuals, which encourages further genetic study of the role of uPAR in cancer.

L13 ANSWER 7 OF 26 MEDLINE on STN

96050896. PubMed ID: 8556526. Role of cytoskeletal elements in expression of monocyte **urokinase** plasminogen activator receptor, activation-associated antigen Mo3. Washington R; Dore-Duffy P. (Department of Neurology, Wayne State University School of Medicine, Detroit, Michigan 48201, USA.) Clinical and diagnostic laboratory immunology, (1994 Nov) 1 (6) 714-21. Journal code: 9421292. ISSN: 1071-412X. Pub. country: United States. Language: English.

AB Peripheral blood monocytes exposed to bacterial products, phorbol esters, cyclic AMP, and cyclic AMP analogs express cell surface activation protein Mo3, which is the **human urokinase plasminogen activator** receptor (uPA-R). uPA-R is expressed by circulating monocytes from patients with multiple sclerosis (MS). We examined the role of cytoskeletal elements in the surface expression and subcellular distribution of uPA-R in nonactivated and lipopolysaccharide-activated monocytes and in monocytes from patients with MS. By using immunofluorescence techniques and confocal laser microscopy, we found that in unactivated monocytes, cytoplasmic uPA-R is found to one side of the nucleus, colocalizing with the Golgi. Upon activation with lipopolysaccharide, cytoplasmic Mo3-uPA-R becomes dispersed throughout the cytoplasm and projections concomitant with an increase in the monocyte perimeter (spreading). Cytoplasmic dispersion, as well as cell surface deposition, is dependent on microtubule integrity. Cell surface deposition of uPA-R upon activation is reduced by colchicine, which disrupts microtubules; however, once associated at the cell surface, uPA-R becomes associated with microfilaments via vinculin. Disruption of microfilaments with cytochalasin also alters surface expression of immunologically reactive uPA-R, as well as the distribution pattern. Monocytes from patients with MS display the uPA-R distribution pattern characteristic of an activated monocyte.

L13 ANSWER 8 OF 26 MEDLINE on STN

96016640. PubMed ID: 8532668. Yeast expression and phagemid display of the **human urokinase plasminogen activator** epidermal growth factor-like domain. Stratton-Thomas J R; Min H Y; Kaufman S E; Chiu C Y; Mullenbach G T; Rosenberg S. (Chiron Corporation, Emeryville, CA 94608, USA.) Protein engineering, (1995 May) 8 (5) 463-70. Journal code: 8801484. ISSN: 0269-2139. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The **human urokinase plasminogen activator** (uPA) epidermal growth factor-like domain (residues 1-48) and a variant with a C-terminal epitope tag have been secreted from recombinant yeast. Purified human uPA 1-48 and uPA 1-48glu complete for binding to the human uPA receptor with Kds of 180 and 400 pM respectively, in an in vitro assay using an immobilized recombinant uPA receptor. A synthetic gene encoding human uPA 1-48 with an N-terminal epitope tag was inserted into a phagemid expression vector as a fusion with residues 249-406 of the M13 pIII protein with an intervening amber codon (TAG). Phagemid production led to infectious particles which were selectively bound and eluted from both epitope tag antibody and **urokinase** receptor. Sequential binding to this antibody and receptor demonstrated a substantial enrichment, where up to 10% of the infectious particles were then retained on **urokinase** receptor-coated plates. A PCR strategy was used to convert previously described peptide bacteriophage ligands for the **urokinase** receptor to phagemid display. The yields of these peptide phagemids and the uPA 1-48 phagemid showed a correlation with peptide affinity, in contrast to when the peptides are multivalently displayed on a bacteriophage.

L13 ANSWER 9 OF 26 MEDLINE on STN

95217327. PubMed ID: 7702750. Regulation of the **urokinase** gene by the retinoblastoma protein. Novak U; Paradiso L; Hamilton J A. (University of Melbourne, Department of Medicine, Royal Melbourne Hospital, Parkville, Victoria, Australia.) DNA and cell biology, (1994 Nov) 13 (11) 1063-9. Journal code: 9004522. ISSN: 1044-5498. Pub. country: United States. Language: English.

AB The promoter of the **human urokinase plasminogen activator** (uPA) gene contains a sequence identical with the retinoblastoma control element (RCE) of the murine c-fos gene, as well as several Sp1 binding sites. In a number of cell lines, the uPA promoter is activated during enforced expression of the retinoblastoma protein, pRB. Electrophoretic mobility-shift assays revealed that the RCE sequence of the uPA gene forms only one specific DNA-protein complex that does not contain pRB. The formation of the RCE-protein complex can be inhibited by 20 molar excess of the unlabeled RCE sequences and by 5 molar excess of the unlabeled E2F binding site. The RCE of the human uPA gene interacts specifically with a protein, which appears to be distinct from members of the E2F family of proteins, Sp1, ATF2, and Elf-1, which are all transcription factors shown to be regulated by pRB.

L13 ANSWER 10 OF 26 MEDLINE on STN

95035933. PubMed ID: 7948815. Chromosomal localization of the **human urokinase plasminogen activator** receptor and plasminogen activator inhibitor type-2 genes: implications in colorectal cancer. Webb G; Baker M S; Nicholl J; Wang Y; Woodrow G; Kruithof E; Doe W F. (Division of Clinical Sciences, John Curtin School of Medical Research, Australian National University, Canberra.) Journal of gastroenterology and hepatology, (1994 Jul-Aug) 9 (4) 340-3. Journal code: 8607909. ISSN: 0815-9319. Pub. country: Australia. Language: English.

AB Activation of the proenzyme of **urokinase** (uPA) on the surface of cancer cells has been implicated in the initiation of focal proteolytic mechanisms that permit invasion and metastasis by colon cancers. The activity of uPA on the cell surface appears to be a function of the number of uPA-specific receptors (uPAR) and the extent of inhibition of uPA by plasminogen activator inhibitors (PAI). The mapping of the genes coding for uPAR, and for PAI-2, was performed to determine whether their chromosomal localization suggested their involvement in the genetic alterations associated with cancer cell DNA. This study confirms the

localization of the **human urokinase plasminogen activator** receptor gene to chromosome 19q and, using in situ hybridization, provides a precise localization to chromosome 19q13.2. In addition, our results confirm the previous allocation of the human plasminogen activator inhibitor-2 gene to a location 18q21.3-->18q22.1, a location that corresponds to the commonest (> 70%) somatic deletions found in colorectal carcinomas. The mapping of the uPAR and PAI-2 genes enables the elucidation of their possible involvement in the genetic alterations that determine the invasive and metastatic phenotypes in colorectal cancer.

L13 ANSWER 11 OF 26 MEDLINE on STN

94350977. PubMed ID: 8071349. Differential DNA sequence specificity and regulation of HIV-1 enhancer activity by cRel-RelA transcription factor. Hansen S K; Guerrini L; Blasi F. (Department of Genetics and Microbiol Biology, University of Milano, Italy.) Journal of biological chemistry, (1994 Sep 2) 269 (35) 22230-7. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The cRel-RelA and NF-kappa B (p50-RelA) transcription factors bind to a kappa B-like sequence termed Rel-related proteins binding element localized in the regulatory region of the **human urokinase plasminogen activator** (uPA) gene. This sequence is highly conserved in murine and porcine uPA genes where it retained the ability to associate with cRel-RelA. On the other hand, NF-kappa B binding was obtained with the human and porcine elements only. Methylation interference analysis showed that NF-kappa B and cRel-RelA had identical interference patterns. Mutational analysis showed that DNA binding was highly sensitive to mutations within the decameric Rel-related proteins binding element core site. However, alterations of nucleotides flanking the decameric IgK-kappa B motif, which preferentially associated with NF-kappa B, resulted in high affinity cRel-RelA binding both in vitro and in vivo. These data demonstrate that NF-kappa B and cRel-RelA have overlapping but distinct DNA sequence specificities. Bandshift analysis with HeLa and Jurkat cell extracts or with in vitro translated proteins revealed that the SV40-, HIV-1-, and interleukin-2 receptor alpha subunit kappa B elements efficiently associated with cRel-RelA, suggesting that this heterodimer may be involved in the regulation of several genes. Cotransfection studies of HIV-1 long terminal repeat-chloramphenicol acetyltransferase reporter DNA with RelA, cRel, and p50 expression vectors were performed in COS7 and U293 cells to analyze the ability of cRel-RelA to regulate HIV-1 enhancer activity. In vivo formation of the cRel-RelA complex resulted in specific stimulation of the viral enhancer at a level comparable with that obtained with NF-kappa B. These data suggest that activation of cellular cRel-RelA may play a critical role in the regulation of HIV-1 enhancer activity.

L13 ANSWER 12 OF 26 MEDLINE on STN

94043730. PubMed ID: 8227331. A ligand-free, soluble **urokinase** receptor is present in the ascitic fluid from patients with ovarian cancer. Pedersen N; Schmitt M; Ronne E; Nicoletti M I; Hoyer-Hansen G; Conese M; Giavazzi R; Dano K; Kuhn W; Janicke F; +. (Institute of Microbiology, University of Copenhagen, Denmark.) Journal of clinical investigation, (1993 Nov) 92 (5) 2160-7. Journal code: 7802877. ISSN: 0021-9738. Pub. country: United States. Language: English.

AB We have identified a soluble form of the **human urokinase plasminogen activator** (uPA) receptor (uPAR) in the ascitic fluids from patients with ovarian cancer. After purification of uPAR from the ascitic fluids by ligand-affinity chromatography (pro-uPA Sepharose), the uPAR was initially identified by cross-linking to a radiolabeled amino-terminal fragment of human uPA. The uPAR purified from the ascitic fluid has no bound ligand (uPA), as similar amounts can be purified by ligand-affinity chromatography as by immuno-affinity chromatography. uPAR from ascitic fluids partitions in the water phase after a temperature-dependent phase separation of a detergent extract. It therefore lacks at least the lipid moiety of the glycophospholipid anchor present in cellular-bound uPARs. It is highly glycosylated and the deglycosylated form has the same electrophoretic mobility as previously characterized cellular uPAR from

other sources. The immunoreactivity of the purified uPAR from the ascitic fluid is indistinguishable from that of characterized uPAR, demonstrated by Western blotting with three different anti-uPAR monoclonal antibodies. The uPAR was found in 11 of 11 ascitic fluids from patients with ovarian cancer and in elevated amounts in the plasma from 2 of 3 patients. The concentration of soluble uPAR in the ascitic fluid was estimated to range between 1 and 10 ng/ml. Human soluble uPAR, derived from the tumor cells, was also found in the ascitic fluid and serum from nude mice xenografted intraperitoneally with three different human ovarian carcinomas.

L13 ANSWER 13 OF 26 MEDLINE on STN

93314820. PubMed ID: 8392005. An alternatively spliced variant of mRNA for the human receptor for **urokinase** plasminogen activator. Pyke C; Eriksen J; Solberg H; Nielsen B S; Kristensen P; Lund L R; Dano K. (Finsen Laboratory, Rigshospitalet, Copenhagen, Denmark.) FEBS letters, (1993 Jul 12) 326 (1-3) 69-74. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB Using 3' RACE (rapid amplification of cDNA ends), we have isolated a cDNA variant for the receptor for **human urokinase plasminogen activator** (uPAR). The deduced protein includes the amino-terminal ligand binding domain in uPAR, but lacks the carboxy-terminal membrane attachment by a glycolipid anchor. Genomic DNA analysis showed that the uPAR mRNA variant is generated by alternative splicing. The new variant mRNA is expressed in various human cell lines and tissues and both variants are up-regulated by phorbol ester in A549 cells. We propose that the alternatively spliced uPAR mRNA encodes a soluble uPA binding protein, the possible function of which is discussed.

L13 ANSWER 14 OF 26 MEDLINE on STN

93252407. PubMed ID: 8098010. The **human urokinase-plasminogen activator** gene (PLAU) is located on chromosome 10q24 centromeric to the HOX11 gene. Stein P M; Stass S A; Kagan J. (Hematopathology Program, University of Texas M.D. Anderson Cancer Center, Houston 77030-4095.) Genomics, (1993 Apr) 16 (1) 301-2. Journal code: 8800135. ISSN: 0888-7543. Pub. country: United States. Language: English.

L13 ANSWER 15 OF 26 MEDLINE on STN

92274957. PubMed ID: 1317293. In situ alkylation of cysteine residues in a hydrophobic membrane protein immobilized on polyvinylidene difluoride membranes by electroblotting prior to microsequence and amino acid analysis. Ploug M; Stoffer B; Jensen A L. (Institute of Biochemical Genetics, University of Copenhagen, Denmark.) Electrophoresis, (1992 Mar) 13 (3) 148-53. Journal code: 8204476. ISSN: 0173-0835. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB For identification of cysteine residues on microsequence analysis it is crucial to derivatize the sulfhydryl groups. This reaction requires a desalting step which often represents a major obstacle, especially if the sample consists of limited amounts of a hydrophobic membrane protein. An alkylation procedure is described, allowing efficient derivatization (greater than 90%) of cysteines and cystines even in low microgram quantities, as revealed by test analyses with lysozyme and a hydrophobic membrane protein. The modified protein is recovered in high yields in a form suitable for both microsequence analysis and amino acid analysis. The method involves electrophoretic desalting by miniaturized Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis and in situ alkylation after electro-transfer onto polyvinylidene difluoride membranes. Precautions against NH2-terminal blocking during sample preparations are provided. The general applicability of the method is illustrated by the structural characterization of the low abundance membrane receptor for **human urokinase plasminogen activator**.

L13 ANSWER 16 OF 26 MEDLINE on STN

91252267. PubMed ID: 1710352. A cell-type specific and enhancer-dependent silencer in the regulation of the expression of the **human urokinase plasminogen activator** gene. Cannio R; Rennie P S; Blasi F. (Institute of Microbiology, University of Copenhagen, Denmark.) Nucleic acids

research, (1991 May 11) 19 (9) 2305 8. Journal code: 0411011. ISSN: 0305-1048. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A transcriptional silencer has been identified in the 5' regulatory region of the **human urokinase plasminogen activator** (uPA) gene. This region is able to block transcription from the human u-PA as well as the rabbit beta-globin promoters in a cell type specific and orientation independent way. The silencer is enhancer dependent and is active in two cell lines (HeLa and CV-1) which produce little if any uPA, but not in the high uPA producer PC3. Silencing activity and enhancer dependence can be separated: the silencing activity has been localized to the DNA fragment -660 to -536, while the enhancer dependence is located in the -536 to -308 fragment. The DNA sequence of the silencer region contains an element that closely resembles the TGF-beta responsive negative element TIE.

L13 ANSWER 17 OF 26 MEDLINE on STN

91224949. PubMed ID: 1851152. A soluble, ligand binding mutant of the **human urokinase plasminogen activator** receptor. Masucci M T; Pedersen N; Blasi F. (Institute of Microbiology, University of Copenhagen, Denmark.) Journal of biological chemistry, (1991 May 15) 266 (14) 8655-8. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB A truncated version of the **human urokinase plasminogen activator** receptor has been obtained by in vitro mutagenesis by insertion of a premature nonsense codon in the **urokinase** plasminogen activator receptor cDNA. This results in a protein truncated immediately upstream of the region which appears to be required for membrane attachment of the receptor via a glycolipid anchor. The modified receptor cDNA inserted into an expression vector has been transfected into mouse LB6 cells. Transfectants produce a **urokinase** plasminogen activator (u-PA)-binding protein that is secreted into the medium. It can be cross-linked to iodinated ATF (amino-terminal fragment of u-PA) and can also inhibit binding of iodinated ATF to mouse LB6 cells that express the wild type human receptor. The soluble u-PA receptor will be used in a variety of experiments aimed at identifying the role and mechanism of u-PA in physiological and pathological invasive processes, as well as in therapeutical attempts to block or decrease cancer cell invasion and in general u-PA-mediated tissue destruction.

L13 ANSWER 18 OF 26 MEDLINE on STN

91200270. PubMed ID: 1901800. Tyrosine phosphorylation of human **urokinase**-type plasminogen activator. Barlati S; Paracini F; Bellotti D; De Petro G. (Department of Biomedical Sciences and Biotechnologies, University of Brescia, Italy.) FEBS letters, (1991 Apr 9) 281 (1-2) 137-40. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB Immunoblotting analysis of purified **human urokinase plasminogen activator** (u-PA), gives a positive signal when reacted with anti-phosphotyrosine monoclonal antibodies (MoAb anti-P-Tyr); competition with o-phospho-DL-tyrosine (P-Tyr) but not o-phospho-DL-threonine or serine (P-Treo, P-Ser) completely suppresses this signal. Either the 55 kDa u-PA form and the lower Mw form (33 kDa) derived from the 55 kDa u-PA are Tyr-phosphorylated also the u-PA secreted in the culture media of human fibrosarcoma cells (HT-1080) is phosphorylated in tyrosine as well as u-PA present in tissue extracts of tumors induced in nude mice by HT-1080 cells. These data show that urine purified human u-PA and u-PA produced by human fibrosarcoma cells, in vitro and in vivo, are phosphorylated in tyrosine; furthermore our data show that u-PA is the major Tyr-phosphorylated protein present in these human tumor cells.

L13 ANSWER 19 OF 26 MEDLINE on STN

90315294. PubMed ID: 2142433. Plasminogen activator mediated degradation of subendothelial extracellular matrix by human squamous carcinoma cell lines. Niedbala M J; Sartorelli A C. (Department of Pharmacology, Yale University School of Medicine, New Haven, CT 06510.) Cancer communications, (1990) 2 (5) 189-99. Journal code: 8916730. ISSN: 0955-3541. Pub. country: United States. Language: English.

AB Extracellular matrix (ECM) produced by bovine corneal endothelial cells was used to investigate the role of the plasminogen activator/plasmin system in the degradation of ECM by human squamous cell carcinoma (SqCCs) and human foreskin epidermal cells (HFEC). SqCCs caused an 8- to 34-fold greater solubilization of 3H-glucosamine-labeled ECM than HFEC. This action in SqCCs was dependent upon the presence of acid-treated serum, indicating that tumor-associated proteinases were sensitive to the inhibitory action of acid-labile proteinase inhibitors present in the serum. SqCC mediated digestion of radiolabeled ECM was decreased by 14- to 55-fold in plasminogen depleted serum, and the addition of 100 micrograms/mL of purified human plasminogen resulted in up to a 30-fold increase in the degradation of the ECM. Inhibitors of this proteinase system and murine monoclonal antibodies (MAb) specific for **human urokinase plasminogen activator** (uPA) decreased the SqCC mediated digestion of radiolabeled ECM in a concentration dependent manner. SqCCs exhibited 10- to 30-fold higher extracellular uPA levels than HFEC, as assayed by substrate hydrolysis, zymography, micro-ELISA, western analysis, and northern analysis. These findings reflect the differential ability of these cell types to degrade the ECM. In addition, immuno-cross-reactive plasminogen activator inhibitor type I (PAI type 1) and type II (PAI type 2) were identified in cell-free conditioned medium produced by both tumor cells and normal epidermal cells, using a micro-ELISA assay. Indirect immunofluorescence flow cytometry, employing MAbs directed against uPA, detected the presence and localization of uPA on the SqCC cell surface. These findings were specific for uPA, since cell surface associated tissue plasminogen activator was not detected in these cell types under analogous conditions. In addition, partially purified SqCC plasma membrane preparations exhibited 2- to 10-fold higher uPA-like activity than HFEC, as determined by zymography. The findings support the concept that the plasminogen activator system is important in the breakdown of ECM by SqCCs and suggest that regulatory mechanisms involved in this proteolytic system may be important targets for chemotherapeutic intervention to limit tumor cell invasion and metastasis.

L13 ANSWER 20 OF 26 MEDLINE on STN

90306332. PubMed ID: 2114315. Serine phosphorylation of biosynthetic pro-**urokinase** from human tumor cells. Mastronicola M R; Stoppelli M P; Migliaccio A; Auricchio F; Blasi F. (International Institute of Genetics and Biophysics, CNR, Naples, Italy.) FEBS letters, (1990 Jun 18) 266 (1-2) 109-14. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB Phosphorylation is a potent mechanism regulating the activity of many intracellular enzymes. We have discovered that the product of the **human urokinase plasminogen activator** gene, pro-uPA, is phosphorylated in serine in at least two human cell lines. Phosphorylation occurs within the cell during biosynthesis, and phosphorylated intracellular pro-uPA is secreted into the medium. Of the secreted pro-uPA molecules, 20-50% are phosphorylated in serine, thus representing a meaningful fraction of the total biosynthetic pro-uPA. Although the sites of phosphorylation have not yet been determined, at least two such sites must exist; in fact plasmin cleavage of phosphorylated single chain pro-uPA yields a two chain uPA in which both chains are phosphorylated. A specific function for pro-uPA phosphorylation has not yet been identified; however, it is tempting to speculate that, as in many other cases, phosphorylation may affect the activity of the enzyme, its response to inhibitors or the conversion of pro-uPA zymogen to active two-chain uPA. This would represent an additional way of regulating extracellular proteolysis, an important pathway involved in both intra- and extravascular phenomena like fibrinolysis, cell migration and invasiveness.

L13 ANSWER 21 OF 26 MEDLINE on STN

90151622. PubMed ID: 1689240. Cloning and expression of the receptor for **human urokinase plasminogen activator**, a central molecule in cell surface, plasmin dependent proteolysis. Roldan A L; Cubellis M V; Masucci M T; Behrendt N; Lund L R; Dano K; Appella E; Blasi F. (University Institute of Microbiology, Copenhagen, Denmark.) EMBO journal, (1990 Feb)

3 (2) 467-74. Journal code: 8200804. ISSN: 0261-4189. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The surface receptor for **urokinase** plasminogen activator (uPAR) has been recognized in recent years as a key molecule in regulating plasminogen mediated extracellular proteolysis. Surface plasminogen activation controls the connections between cells, basement membrane and extracellular matrix, and therefore the capacity of cells to migrate and invade neighboring tissues. We have isolated a 1.4 kb cDNA clone coding for the entire human uPAR. An oligonucleotide synthesized on the basis of the N-terminal sequence of the purified protein was used to screen a cDNA library made from SV40 transformed human fibroblasts [Okayama and Berg (1983) Mol. Cell Biol., 3, 280-289]. The cDNA encodes a protein of 313 amino acids, preceded by a 21 residue signal peptide. A hydrophobicity plot suggests the presence of a membrane spanning domain close to the C-terminus. The cDNA hybridizes to a 1.4 kb mRNA from human cells, a size very close to that of the cloned cDNA. Expression of the uPAR cDNA in mouse cells confirms that the clone is complete and expresses a functional uPA binding protein, located on the cell surface and with properties similar to the human uPAR. Caseinolytic plaque assay, immunofluorescence analysis, direct binding studies and cross-linking experiments show that the transfected mouse LB6 cells specifically bind human uPA, which in turn activates plasminogen. The Mr of the mature human receptor expressed in mouse cells is approximately 55,000, in accordance with the naturally occurring, highly glycosylated human uPAR. The Mr calculated on the basis of the cDNA sequence, approximately 35,000, agrees well with that of the deglycosylated receptor.

L13 ANSWER 22 OF 26 MEDLINE on STN

89083492. PubMed ID: 3205721. An upstream enhancer and a negative element in the 5' flanking region of the **human urokinase plasminogen activator** gene. Verde P; Boast S; Franze A; Robbiati F; Blasi F. (International Institute of Genetics and Biophysics, CNR, Naples, Italy.) Nucleic acids research, (1988 Nov 25) 16 (22) 10699-716. Journal code: 0411011. ISSN: 0305-1048. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The 5' flanking region of the human **urokinase** (uPA) gene has been fused to the reporter chloramphenicol acetyl transferase (CAT) gene and its activity assayed by transfection in two human cell lines. Progressive deletions of the uPA regulatory region from the 5' end maintain a high level of expression provided at least 1870 (in A1251 cells) or 1963 (in HFS10 cells) nucleotides of the 5' flanking region are retained. A DNA fragment from -2350 to -1824 has enhancer properties, stimulating transcription of an enhancerless SV40 early promoter independently of orientation and distance. Internal deletions that still retain the enhancer element reveal the presence of negative cis-acting sequences between -1824 and -1572. Their removal, in fact, increases uPA transcriptional activity. Differences of expression of the uPA-CAT fusion genes in the two cell lines are also observed, indicating the presence of cell-specific cis-acting sequences.

L13 ANSWER 23 OF 26 MEDLINE on STN

87121003. PubMed ID: 2433787. A monoclonal antibody that recognizes the receptor binding region of **human urokinase plasminogen activator**. Nolli M L; Corti A; Soffientini A; Cassani G. Thrombosis and haemostasis, (1986 Oct 21) 56 (2) 214-8. Journal code: 7608063. ISSN: 0340-6245. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.

AB An anti-**urokinase** monoclonal antibody 5B4 (MAB 5B4) was obtained by fusing the murine myeloma cell line X63-Ag8.653 with the spleen cells from a female BALB/c mouse immunized with high-molecular-weight **urokinase** (HMW-uPA). MAB 5B4 is an IgG1 that binds selectively to the single-chain form of uPA (sc-uPA), to HMW-uPA and to the 17,000 Mr aminoterminal fragment of the A-chain (ATF) but not to the low-molecular-weight **urokinase** (LMW-uPA) nor to the reduced form of HMW-uPA. This strongly suggests that MAB 5B4 recognizes a conformational determinant on the A-chain. The antibody has an affinity constant for uPA-Sepharose of 1.42 X 10(7) M-1, calculated from equilibrium binding data, and can be used for

one step purification of HMW uPA by immunoaffinity chromatography. MAB 5B4 and the previously obtained antibody 105IF10 recognize the A-chain: the epitopes, however, are distinct as shown by double-antibody-sandwich enzyme immunoassay. Finally MAB 5B4 inhibits the binding of ATF to the uPA receptor of different human cells, whereas 105IF10 does not. Thus this antibody represents a potentially, useful tool for the study of uPA receptor physiology.

L13 ANSWER 24 OF 26 MEDLINE on STN

87057228. PubMed ID: 3023326. Binding of single-chain prourokinase to the **urokinase** receptor of human U937 cells. Cubellis M V; Nolli M L; Cassani G; Blasi F. Journal of biological chemistry, (1986 Dec 5) 261 (34) 15819-22. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The single-chain form of **human urokinase plasminogen activator** (uPA) is the major form of the enzyme found in cells, tissues, and extracellular fluids. The protein, called pro-uPA, has high (Kd = 0.5 nM) affinity for the specific uPA receptor of U937 human monocyte-like cells. Its conversion to two-chain uPA by plasmin does not appreciably change the binding parameters. In addition, conversion of pro-uPA to uPA occurs with receptor-bound pro-uPA and does not lead to dissociation from the membrane. These data show that secreted pro-uPA can find its way to the specific surface receptor without previous conversion to the two-chain form and that, once bound, can be activated by plasmin.

L13 ANSWER 25 OF 26 MEDLINE on STN

85270442. PubMed ID: 2991901. Differentiation-enhanced binding of the amino-terminal fragment of **human urokinase plasminogen activator** to a specific receptor on U937 monocytes. Stoppelli M P; Corti A; Soffientini A; Cassani G; Blasi F; Assoian R K. Proceedings of the National Academy of Sciences of the United States of America, (1985 Aug) 82 (15) 4939-43. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB The purified amino-terminal fragment (ATF) of **human urokinase plasminogen activator** (residues 1-135), which is not required for activation of plasminogen, binds with high affinity to specific plasma membrane receptors on U937 monocytes. Intact **urokinase** efficiently competes for 125I-labeled ATF binding; 50% competition occurs with 1 nM **urokinase**. A large part of receptor-bound **urokinase** remains on the cell surface for at least 2 hr at 37 degrees C. Differentiation of U937 monocytes into macrophage-like cells specifically increases ATF binding 10- to 20-fold. These results suggest an important role for **urokinase** in monocyte/macrophage biology: the native enzyme binds to the cells with the amino-terminal domain; the catalytic, carboxyl-terminal domain remains exposed on the cell surface to stimulate localized proteolysis and facilitate cell migration.

L13 ANSWER 26 OF 26 MEDLINE on STN

85215647. PubMed ID: 2987867. The **human urokinase-plasminogen activator** gene and its promoter. Riccio A; Grimaldi G; Verde P; Sebastio G; Boast S; Blasi F. Nucleic acids research, (1985 Apr 25) 13 (8) 2759-71. Journal code: 0411011. ISSN: 0305-1048. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The **urokinase** type of plasminogen activator (uPA) is subject to regulation by hormones, phorbol esters and oncogenic transformation. This enzyme has been suggested to play a key role in processes involving cell migration and tissue remodeling, and to be essential for tumor metastasis. In order to study these processes, we have isolated the human uPA gene, and have determined its entire nucleotide sequence. The gene is organized in 11 exons and is 6.4 kb long. The 5' end of uPA mRNA has been determined by both S1 mapping and primer extension experiments. A fragment of 800 bp containing the entire 5' flanking region shows promoter activity when introduced upstream of a bacterial chloramphenicol acetyltransferase gene and introduced into human cells. The hexanucleotide sequence GGCGGG, previously found at similar regions in several viral and eukaryotic promoters and shown to be essential for

promoter activity (McKnight et al. (1984) Cell, 37, 253-262), is repeated three times between the CAAT and the TATA boxes.

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=> d his

     (FILE 'HOME' ENTERED AT 22:23:33 ON 10 MAR 2004)

     FILE 'USPATFULL' ENTERED AT 22:24:23 ON 10 MAR 2004
                E WADA MANABU/IN
L1            7 S E3
                E WADA NAOKO/IN
L2            2 S E3

     FILE 'MEDLINE' ENTERED AT 22:25:28 ON 10 MAR 2004
                E WADA M/AU
L3          875 S E3
L4            2 S L3 AND (UROKINASE)
                E WADA N/AU
L5            3 S E10

     FILE 'USPATFULL' ENTERED AT 22:28:52 ON 10 MAR 2004
L6         6244 S UROKINASE
L7           38 S L6 AND (HUMAN UROKINASE PLASMINOGEN ACTIVATOR)
L8            2 S L7 AND (HUMAN UROKINASE PLASMINOGEN ACTIVATOR/CLM)
L9           36 S L7 NOT L8
L10         166 S L6 AND (ATF OR AMINO-TERMINAL FRAGMENT)
L11          13 S L10 AND (ATF/CLM OR AMINO-TERMINAL FRAGMENT/CLM)

     FILE 'MEDLINE' ENTERED AT 22:38:16 ON 10 MAR 2004
L12        9092 S (UROKINASE OR HUMAN UROKINASE PLASMINOGEN ACTIVATOR)
L13          26 S L12 AND (HUMAN UROKINASE PLASMINOGEN ACTIVATOR)

=> s l12 and (ATF or amino terminal fragment)
          1980 ATF
        549473 AMINO
        205246 TERMINAL
         99118 FRAGMENT
           491 AMINO TERMINAL FRAGMENT
                 (AMINO(W)TERMINAL(W)FRAGMENT)
L14          133 L12 AND (ATF OR AMINO TERMINAL FRAGMENT)

=> s l14 not l13
L15          129 L14 NOT L13

=> d l15,ti,100-129

L15  ANSWER 100 OF 129     MEDLINE on STN
TI   Solution structure of the amino-terminal fragment of
     urokinase-type plasminogen activator.

L15  ANSWER 101 OF 129     MEDLINE on STN
TI   Requirement for receptor-bound urokinase in plasmin-dependent cellular
     conversion of latent TGF-beta to TGF-beta.

L15  ANSWER 102 OF 129     MEDLINE on STN
TI   Low density lipoprotein receptor-related protein/alpha 2-macroglobulin
     receptor mediates cellular uptake of pro-urokinase.

L15  ANSWER 103 OF 129     MEDLINE on STN
TI   Processing of complex between urokinase and its type-2 inhibitor on the
     cell surface. A possible regulatory mechanism of urokinase activity.

L15  ANSWER 104 OF 129     MEDLINE on STN
TI   Purification and cDNA cloning of a transcription factor which functionally
     cooperates within a cAMP regulatory unit in the porcine uPA gene.
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L15 ANSWER 105 OF 129 MEDLINE on STN
TI Saturation of tumour cell surface receptors for **urokinase**-type plasminogen activator by **amino-terminal fragment** and subsequent effect on reconstituted basement membranes invasion.

L15 ANSWER 106 OF 129 MEDLINE on STN
TI **Urokinase-urokinase** receptor interaction: non-mitogenic signal transduction in human epidermal cells.

L15 ANSWER 107 OF 129 MEDLINE on STN
TI Selective localization of receptors for **urokinase amino-terminal fragment** at substratum contact sites of an in vitro-established line of human epidermal cells.

L15 ANSWER 108 OF 129 MEDLINE on STN
TI Heparin binding to the **urokinase** kringle domain.

L15 ANSWER 109 OF 129 MEDLINE on STN
TI **Urokinase** binding to laminin-nidogen. Structural requirements and interactions with heparin.

L15 ANSWER 110 OF 129 MEDLINE on STN
TI Purified alpha 2-macroglobulin receptor/LDL receptor-related protein binds **urokinase**.plasminogen activator inhibitor type-1 complex. Evidence that the alpha 2-macroglobulin receptor mediates cellular degradation of **urokinase** receptor-bound complexes.

L15 ANSWER 111 OF 129 MEDLINE on STN
TI Structural requirements for the growth factor activity of the amino-terminal domain of **urokinase**.

L15 ANSWER 112 OF 129 MEDLINE on STN
TI Internalization of the **urokinase**-plasminogen activator inhibitor type-1 complex is mediated by the **urokinase** receptor.

L15 ANSWER 113 OF 129 MEDLINE on STN
TI Demonstration of a specific clearance receptor for tissue-type plasminogen activator on rat Novikoff hepatoma cells.

L15 ANSWER 114 OF 129 MEDLINE on STN
TI Biological and clinical relevance of the **urokinase**-type plasminogen activator (uPA) in breast cancer.

L15 ANSWER 115 OF 129 MEDLINE on STN
TI **Urokinase**-type plasminogen activator mediates basic fibroblast growth factor-induced bovine endothelial cell migration independent of its proteolytic activity.

L15 ANSWER 116 OF 129 MEDLINE on STN
TI An autocrine role for **urokinase** in phorbol ester-mediated differentiation of myeloid cell lines.

L15 ANSWER 117 OF 129 MEDLINE on STN
TI Cellular receptor for **urokinase** plasminogen activator. Carboxyl-terminal processing and membrane anchoring by glycosyl-phosphatidylinositol.

L15 ANSWER 118 OF 129 MEDLINE on STN
TI An **amino-terminal fragment** of **urokinase** isolated from a prostate cancer cell line (PC-3) is mitogenic for osteoblast-like cells.

L15 ANSWER 119 OF 129 MEDLINE on STN
TI Alveolar macrophage **urokinase** receptors localize enzyme activity to the cell surface.

L15 ANSWER 120 OF 129 MEDLINE on STN

TI Receptor mediated internalization and degradation of **urokinase** is caused by its specific inhibitor PAI-1.

L15 ANSWER 121 OF 129 MEDLINE on STN
TI The human receptor for **urokinase** plasminogen activator. NH2-terminal amino acid sequence and glycosylation variants.

L15 ANSWER 122 OF 129 MEDLINE on STN
TI Localization of **urokinase**-type plasminogen activator receptor on U937 cells: phorbol ester PMA induces heterogeneity.

L15 ANSWER 123 OF 129 MEDLINE on STN
TI Interaction of single-chain **urokinase**-type plasminogen activator with human endothelial cells.

L15 ANSWER 124 OF 129 MEDLINE on STN
TI Epitope mapping of the anti-**urokinase** monoclonal antibody 5B4 by isolated domains of **urokinase**.

L15 ANSWER 125 OF 129 MEDLINE on STN
TI Accessibility of receptor-bound **urokinase** to type-1 plasminogen activator inhibitor.

L15 ANSWER 126 OF 129 MEDLINE on STN
TI Plasminogen activation initiated by single-chain **urokinase**-type plasminogen activator. Potentiation by U937 monocytes.

L15 ANSWER 127 OF 129 MEDLINE on STN
TI A 55,000-60,000 Mr receptor protein for **urokinase**-type plasminogen activator. Identification in human tumor cell lines and partial purification.

L15 ANSWER 128 OF 129 MEDLINE on STN
TI Sensitive and specific enzyme-linked immunosorbent assay for **urokinase**-type plasminogen activator and its application to plasma from patients with breast cancer.

L15 ANSWER 129 OF 129 MEDLINE on STN
TI The receptor-binding sequence of **urokinase**. A biological function for the growth-factor module of proteases.

=> d l15,cbib,ab,1-129

L15 ANSWER 1 OF 129 MEDLINE on STN
2004054847. PubMed ID: 14749538. Differential inhibition of endothelial cell proliferation and migration by **urokinase** subdomains: **amino-terminal fragment** and kringle domain. Kim Kwang Sei; Hong Yong-Kil; Lee Yoon; Shin Joo-Young; Chang Soo-Ik; Chung Soo Il; Joe Young Ae. (Cancer Research Institute, Catholic Research Institute of Medicial Sciences, The Catholic University of Korea, Seoul 137-701, Korea.) Experimental & molecular medicine, (2003 Dec 31) 35 (6) 578-85. Journal code: 9607880. ISSN: 1226-3613. Pub. country: Korea (South). Language: English.
AB The serine protease **urokinase**-type plasminogen activator (uPA) is implicated in pericellular proteolysis in a variety of physiological and pathological processes including angiogenesis and tumor metastasis. The kringle domain of uPA (UK1) has proven to be an anti-angiogenic molecule with unknown mechanism and **amino terminal fragment** of uPA (u-**ATF**) with additional growth factor-like domain can be used for blocking interaction of uPA and uPA receptor. Here, we compared anti-angiogenic activities of these two molecules in vitro and in vivo. The recombinant u-**ATF** from E. coli and refolded in vitro was found to bind to uPAR with high affinity, whereas E. coli-derived UK1 showed no binding by Biacore analysis. In contrast to UK1 having potent inhibitory effect, u-**ATF** exhibited low inhibitory effect on bovine capillary endothelial cell

growth (ED(50)/320 nM). Furthermore, a **ATF** inhibition of VEGF induced migration of human umbilical vein endothelial cell was far less sensitive (IC(50) = 600 nM) than those observed with UK1, and angiogenesis inhibition was marginal in chorioallantoic membrane. These results suggest that kringle domain alone is sufficient for potent anti-angiogenic activity and additional growth factor-like domain diverts this molecule in undergoing different mechanism such as inhibition of uPA/uPAR interaction rather than undergoing distinct anti- angiogenic mechanism driven by kringle domain.

L15 ANSWER 2 OF 129 MEDLINE on STN

2004052103. PubMed ID: 14720519. EGF receptor transactivation by **urokinase** receptor stimulus through a mechanism involving Src and matrix metalloproteinases. Guerrero Javier; Santibanez Juan Francisco; Gonzalez Alfonso; Martinez Jorge. (Laboratorio de Biologia Celular, INTA, Universidad de Chile, Santiago 11, Chile.) Experimental cell research, (2004 Jan 1) 292 (1) 201-8. Journal code: 0373226. ISSN: 0014-4827. Pub. country: United States. Language: English.

AB **Urokinase**-type plasminogen activator receptor (uPAR) and epidermal growth factor receptor (EGFR) are ubiquitous receptors involved in the control of a variety of cellular processes frequently found altered in cancer cells. The EGFR has been recently described to play a transduction role of uPAR stimuli, mediating uPA-induced proliferation in highly malignant cells that overexpress uPAR. In the present work, we found for the first time that uPAR stimulation with the **amino-terminal fragment** (**ATF**) of **urokinase** devoid of proteolytic activity transactivates the EGFR in mammary MCF-7 cells through a mechanism involving Src and a metalloproteinase, as indicated by its sensitivity to selected inhibitors. In these cells, which express low levels of uPAR and malignancy, both **ATF** and EGF stimuli induced an interaction of the EGFR with uPAR and ERK activation. However, EGFR activation by uPAR stimuli mediated cellular invasion rather than proliferation, while EGFR activation by EGF led to a proliferative response. These results revealed a complex modulation of EGFR function toward different cellular responses according to the status of uPAR activity. On the other hand, we also found that MMP-mediated activation of EGFR can occur in an autocrine manner in cells which secrete uPA. All this reveals novel regulatory systems operating through autocrine loops involving uPAR stimuli, Src, MMP and EGFR activation which could mediate fine control of physiological processes as well as contribute to the expression of proliferative and invasive phenotypes of cancerous cells.

L15 ANSWER 3 OF 129 MEDLINE on STN

2004021426. PubMed ID: 14718842. Domain-dependent action of **urokinase** on smooth muscle cell responses. Tanski William J; Fegley Allison J; Roztocil Elisa; Davies Mark G. (Department of Surgery, University of Rochester, Strong Memorial Hospital, Box SURG, 601 Elmwood Avenue, Rochester, NY 14642, USA.. wtanski@rochester.rr.com) . Journal of vascular surgery : official publication, Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter, (2004 Jan) 39 (1) 214-22. Journal code: 8407742. ISSN: 0741-5214. Pub. country: United States. Language: English.

AB BACKGROUND: Single-chain **urokinase**-type plasminogen activator (sc-uPA) is one of the key serine proteases involved in modulating cellular and extracellular matrix responses during tissue remodeling. Sc-uPA is composed of three domains: aminoterminal fragment (**ATF**), kringle domain, and carboxyterminal fragment (CTF). sc-uPA is readily cleaved into these three domain fragments in vitro, each of which is biologically active; however, their roles in the microenvironment of the vessel wall are poorly understood. PURPOSE: The purpose of this study was to determine the role of each domain of sc-uPA on vascular smooth muscle cell (SMC) proliferation and migration. METHODS: SMCs were cultured in vitro. Assays of DNA synthesis, cell proliferation, and migration were performed in response to sc-uPA, **ATF**, kringle, and CTF in the presence and absence of the plasmin inhibitors epsilon-aminocaproic acid (EACA) and aprotinin, the Galphai inhibitor pertussis toxin, and the mitogen-activated protein

kinase 1 (the upstream regulator of the extracellular signal regulated kinase [ERK]) inhibitor PD98059. RESULTS: sc-uPA produced dose-dependent increases in DNA synthesis and cell proliferation. These responses were dependent on the CTF domain and were sensitive to plasmin inhibitors, pertussis toxin, and PD98059. Sc-uPA also induced SMC migration, which could be elicited by both **ATF** and kringle. Migration to sc-uPA, **ATF**, and kringle was both pertussis toxin and PD98059 sensitive, but importantly was plasmin-independent. CONCLUSION: sc-uPA induces SMC proliferation and migration, which are domain-dependent and mediated in part by Galphai-linked, ERK-dependent processes, while only the mitogenic response is protease dependent. These findings suggest that migration is linked to a G-protein coupled nonprotease receptor, while proliferation is associated with a G-protein coupled protease receptor.

L15 ANSWER 4 OF 129 MEDLINE on STN

2003562324. PubMed ID: 14649886. Targeting the over-expressed **urokinase**-type plasminogen activator receptor on glioblastoma multiforme. Rustamzadeh Edward; Li Chunbin; Doumbia Sekou; Hall Walter A; Vallera Daniel A. (Department of Neurosurgery, University of Minnesota Cancer Center, Minneapolis, MN 55455, USA.) Journal of neuro-oncology, (2003 Oct) 65 (1) 63-75. Ref: 143. Journal code: 8309335. ISSN: 0167-594X. Pub. country: Netherlands. Language: English.

AB A recombinant fusion protein targeting the **urokinase**-type plasminogen activator receptor (uPAR) and delivering a potent catalytic toxin has the advantage of simultaneously targeting both over-expressed uPAR on glioblastoma cells and on the tumor neovasculature. Such a hybrid protein was synthesized consisting of the noninternalizing **amino-terminal fragment** (**ATF**) of **urokinase**-type plasminogen activator (uPA) for binding, and the catalytic portion of diphtheria toxin (DT) for killing, and the translocation enhancing region (TER) of DT for internalization. The protein was highly selective for human glioblastoma in vitro and in vivo. In vivo, this DT/**ATF** hybrid called DTAT caused the regression of small subcutaneous uPAR-expressing tumors with minimal toxicity to critical organs. In vitro, DTAT killed only uPAR-positive glioblastoma cell lines and human endothelial cells in the form of the HUVEC cell line. Killing was selective and blockable with specific antibody. DTAT was highly effective against tumor cells cultured from glioblastoma multiforme patients and in vitro mixing experiments combining DTAT with DTIL13 another highly effective anti-glioblastoma agent showed that the mixture was as toxic as the most potent immunotoxin. In this article, we review our progress to date with DTAT.

L15 ANSWER 5 OF 129 MEDLINE on STN

2003518914. PubMed ID: 12881310. The kringle stabilizes **urokinase** binding to the **urokinase** receptor. Bdeir Khalil; Kuo Alice; Sachais Bruce S; Rux Ann H; Bdeir Yasmina; Mazar Andrew; Higazi Abd Al-Roof; Cines Douglas B. (Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA.) Blood, (2003 Nov 15) 102 (10) 3600-8. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB The structural basis of the interaction between single-chain **urokinase**-type plasminogen activator (scuPA) and its receptor (uPAR) is incompletely defined. Several observations indicated the kringle facilitates the binding of uPA to uPAR. A scuPA variant lacking the kringle (Delta K-scuPA) bound to soluble uPAR (suPAR) with the similar "on-rate" but with a faster "off-rate" than wild-type (WT)-scuPA. Binding of Delta K-scuPA, but not WT-scuPA, to suPAR was comparably inhibited by its growth factor domain (GFD) and **amino-terminal fragment** (**ATF**). **ATF** and WT-scuPA, but not GFD, scuPA lacking the GFD (Delta GFD-scuPA), or Delta K-scuPA reconstituted the isolated domains of uPAR. **ATF** completely inhibited the enzymatic activity of WT-scuPA-suPAR unlike comparable concentrations of GFD. Variants containing mutations that alter the charge, length, or flexibility of linker sequence (residues 43-49) between the GFD and the kringle displayed a lower affinity for uPAR, were unable to reconstitute uPAR domains, and their binding to uPAR was inhibited by GFD in the same manner as Delta K-scuPA. A scuPA variant

in which the charged amino acids in the heparin binding site (HBS) in the kringle domain were mutated to alanines behaved like Delta K-scuPA, indicating that that the structure of the kringle as well as its interaction with the GFD govern receptor binding. These data demonstrate an important role for the kringle in stabilizing the binding of scuPA to uPAR.

L15 ANSWER 6 OF 129 MEDLINE on STN

2003517119. PubMed ID: 12960238. The role of **urokinase**-type plasminogen activator (uPA)/uPA receptor in HIV-1 infection. Alfano Massimo; Sidenius Nicolai; Blasi Francesco; Poli Guido. (Department of Immunology and Infectious Disease, Vita-Salute University School of Medicine, Milan, Italy.) Journal of leukocyte biology, (2003 Nov) 74 (5) 750-6. Ref: 82. Journal code: 8405628. ISSN: 0741-5400. Pub. country: United States. Language: English.

AB The binding of **urokinase**-type plasminogen activator (uPA) to its glycosyl-phosphatidyl-inositol (GPI) anchored receptor (uPAR) mediates a variety of functions in terms of vascular homeostasis, inflammation and tissue repair. Both uPA and uPAR, as well as their soluble forms detectable in plasma and other body fluids, represent markers of cancer development and metastasis, and they have been recently described as predictors of human immunodeficiency virus (HIV) disease progression, independent of CD4+ T cell counts and viremia. A direct link between the uPA/uPAR system and HIV infection was earlier proposed in terms of cleavage of gp120 envelope by uPA. More recently, a negative regulatory effect on both acutely and chronically infected cells has been linked to the noncatalytic portion of uPA, also referred to as the **amino-terminal fragment (ATF)**. **ATF** has also been described as a major CD8+ T cell soluble HIV suppressor factor. In chronically infected promonocytic U1 cells this inhibitory effect is exerted at the very late stages of the virus life cycle, involving virion budding and entrapment in intracytoplasmic vacuoles, whereas its mechanism of action in acutely infected cells remains to be defined. Since uPAR is a GPI-anchored receptor it requires association with a signaling-transducing component and different partners, which include CD11b/CD18 integrin and a G-protein coupled receptor homologous to that for the bacterial chemotactic peptide formyl-methionyl-leucyl-phenylalanine. Which signaling coreceptor(s) is(are) responsible for uPA-dependent anti-HIV effect remains currently undefined.

L15 ANSWER 7 OF 129 MEDLINE on STN

2003479422. PubMed ID: 14557634. Formation of polyomavirus-like particles with different VP1 molecules that bind the **urokinase** plasminogen activator receptor. Shin Young C; Folk William R. (Department of Biochemistry, University of Missouri-Columbia, Columbia, Missouri 65211, USA.) Journal of virology, (2003 Nov) 77 (21) 11491-8. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB Icosahedral virus-like particles formed by the self-assembly of polyomavirus capsid proteins (Py-VLPs) can serve as useful nanostructures for delivering nucleic acids, proteins, and pharmaceuticals into animal cells and tissues. Four predominant surface-exposed loops in the VP1 structure offer potential sites to display sequences that might contribute new targeting specificities. Introduction into each of these loops of sequences derived from the **amino-terminal fragment** of **urokinase** plasminogen activator (uPA) or a related phage display peptide reduced the solubility of VP1 molecules when expressed in insect cells, and insertions into the EF loop reduced VP1 solubility least. Coexpression in insect cells of the uPA-VP1 molecules and VP1 containing a FLAG epitope in the HI loop permitted the formation of heterotypic Py-VLPs containing uPA-VP1 and FLAG-VP1. These heterotypic VLPs bound to uPAR on the surfaces of animal cells. Heterotypic Py-VLPs containing ligands for multiple cell surface receptors should be useful for targeting specific cells and tissues.

L15 ANSWER 8 OF 129 MEDLINE on STN

2003472169. PubMed ID: 12827296. Targeting of a novel fusion protein containing methioninase to the **urokinase** receptor to inhibit breast

cancer cell migration and proliferation. Felon Kaline, Jones Tara N; Gauthier Sebastien A; Nguyen Thao-Nguyen T; Zang Xiao-Ping; Barriere Magali; Preveraud Damien; Soliman Charles E; Harrison Roger G; Pento J Thomas. (Bioengineering Center and the School of Chemical Engineering and Materials Science, University of Oklahoma, Norman, Oklahoma 73019, USA.) Cancer chemotherapy and pharmacology, (2003 Oct) 52 (4) 270-6. Journal code: 7806519. ISSN: 0344-5704. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB It has been shown that methionine depletion inhibits tumor cell growth and reduces tumor cell survival. A novel fusion protein targeted specifically to tumor cells was developed. The fusion protein contained two components: the **amino terminal fragment** of human **urokinase** (amino acids 1-49) that binds to the **urokinase** receptor protein expressed on the surface of invasive cancer cells, and the enzyme L-methioninase (containing 398 amino acids) which depletes methionine and arrests the growth of methionine-dependent tumors. The influence of the fusion protein on the growth and motility of human breast cancer cells was examined using a culture wounding assay. It was determined that MCF-7 breast cancer cells, used in this study, were methionine-dependent and that the fusion protein bound specifically to **urokinase** receptors of the surface of the cancer cells. Further treatment of the cancer cells with fusion protein over the concentration range 10(-8) to 10(-6) M produced a dose-dependent inhibition of both the migration and proliferation index of MCF-7 cells in the culture wounding assay over a period of 1 to 3 days. The results of this study suggest that this novel fusion protein may serve as a prototype for specific targeting of methioninase and perhaps other cytotoxic agents to cancer cells.

L15 ANSWER 9 OF 129 MEDLINE on STN

2003351754. PubMed ID: 12883632. Biological function of fusion protein **ATF**-PAI2CD. Wang Xia; Li Ping; Zhang Yu-Qing; Hou Min; Sun Xing-Hui; Tan Li; Zhu Yun-Song. (Department of Molecular Genetics, Shanghai Medical College, Fudan University, Shanghai 200032, China.) Sheng wu hua xue yu sheng wu wu li xue bao Acta biochimica et biophysica Sinica, (2003 Jul) 35 (7) 624-8. Journal code: 20730160R. ISSN: 0582-9879. Pub. country: China. Language: Chinese.

AB To express the fusion protein **ATF**-PAI2CD (**urokinase**-type plasminogen activator **amino terminal fragment**-plasminogen activator inhibitor type 2 with the region inter C and D helices deleted) gene in E.coli and determine the biological characterization of fusion protein **ATF**-PAI2CD, the cDNA fragment encoding **ATF**-PAI2CD was cloned into the expression vector pLY-4 and transformed into E.coli JF1125. After temperature induction, the expression amount of **ATF**-PAI2CD account for 15% of total bacterial protein. The result was confirmed by Western blot. **ATF**-PAI2CD protein was isolated and purified by washing and solubilization of inclusion body, renaturation and ion exchange chromatography. The final product displayed a single band with a corresponding molecular weight 62 kD in SDS-PAGE. The purity was over 90%, the protein yield was 50% and the specific activity was 12 000 IU/mg. The PAI activity was measured by chromogenic assay. The purified fusion protein inhibited **urokinase**-type plasminogen activator as measured by milk-agarose plate assay, and bound to human lung cancer cells via uPA receptor (uPAR), which was confirmed by radio competition experiments. The results indicate that the biological characteristics of **ATF**-PAI2CD were very similar to those of the wide type PAI-2 (or mutants PAI-2, PAI-2CD) and to pro-uPA in binding to uPAR-bearing cells.

L15 ANSWER 10 OF 129 MEDLINE on STN

2003279414. PubMed ID: 12729802. Noncatalytic domain of uPA stimulates human extravillous trophoblast migration by using phospholipase C, phosphatidylinositol 3-kinase and mitogen-activated protein kinase. Liu Jessica; Chakraborty Chandan; Graham Charles H; Barbin Youssef P; Dixon S Jeffrey; Lala Peeyush K. (Department of Anatomy and Cell Biology, Medical Sciences Building, Faculty of Medicine and Dentistry, University of Western Ontario, London, Ontario, Canada N6A 5C1.) Experimental cell research, (2003 May 15) 286 (1) 138-51. Journal code: 0373226. ISSN:

0014-4827. Pub. country: United States. Language: English.

AB The serine protease **urokinase**-type plasminogen activator (uPA) promotes matrix degradation by many cell types, including the invasive extravillous trophoblast (EVT) of the human placenta. The noncatalytic amino-terminal end of uPA binds to uPA receptors (uPARs) expressed by these cells. A highly polarized expression of uPAR-bound uPA at the migration front of EVT cells in situ suggests a functional role of uPA:uPAR interaction in EVT cell migration. The present study examined whether uPA stimulates EVT cell migration, independent of proteolytic function, and investigated some of the signaling pathways involved. Using in vitro-propagated EVT cells in Transwell migration assays, both uPA and its noncatalytic **amino-terminal fragment** (**ATF**) were shown to stimulate migration through multiporous polycarbonate (pore size 8 microm) membranes. A uPAR-blocking antibody inhibited basal and **ATF**-stimulated migration. Migration was found to be stimulated by hypoxic conditions, which upregulates uPAR expression; this stimulation was abrogated with the uPAR-blocking antibody, indicating the role of endogenous uPA in EVT cell migration. Spectrofluorometric measurement of cytosolic calcium in cells treated with uPA and **ATF** demonstrated a rapid rise in [Ca2+](i), which was prevented by pretreatment of cells with thapsigargin, indicating a release from intracellular stores. Both basal and **ATF**-mediated migratory responses were suppressed in the presence of selective pharmacological inhibitors LY294002, U73122, and U0126, implicating the respective roles of phosphatidinylinositol 3-kinase (PI 3-K), phospholipase C (PLC), and MEK1/2 in basal and **ATF**-stimulated migratory capacity. Taken together, these results demonstrate that uPA:uPAR interaction stimulates EVT cell migration, independent of uPA enzymatic activity, using the mitogen-activated protein kinase pathway and calcium signaling events including the participation of PI 3-K and PLC. These findings are relevant to clinical conditions of aberrant trophoblast migration, including spontaneous abortion, preeclampsia, and choriocarcinoma.

L15 ANSWER 11 OF 129 MEDLINE on STN

2003219227. PubMed ID: 12642587. Induction of plasminogen activator inhibitor-1 by **urokinase** in lung epithelial cells. Shetty Sreerama; Bdeir Khalil; Cines Douglas B; Idell Steven. (Department of Specialty Care Services, University of Texas Health Center, Tyler, Texas 75708, USA.) Journal of biological chemistry, (2003 May 16) 278 (20) 18124-31. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The plasminogen/plasmin system, **urokinase**-type plasminogen activator (uPA), its receptor (uPAR), and its inhibitor (PAI-1), influence extracellular proteolysis and cell migration in lung injury or neoplasia. In this study, we sought to determine whether tcuPA (two chain uPA) alters expression of its major inhibitor PAI-1 in lung epithelial cells. The expression of PAI-1 was evaluated at the protein and mRNA level by Western blot, immunoprecipitation, and Northern blot analyses. We found that tcuPA treatment enhanced PAI-1 protein and mRNA expression in Beas2B lung epithelial cells in a time- and concentration-dependent manner. The tcuPA-mediated induction of PAI-1 involves post-transcriptional control involving stabilization of PAI-1 mRNA. Inactivation of the catalytic activity of tcuPA had little effect on PAI-1 induction and the activity of the isolated **amino-terminal fragment** was comparable with full-length single- or two-chain uPA. In contrast, deletion of either the uPA receptor binding growth factor domain or kringle domain (kringle) from full-length single chain uPA markedly attenuated the induction of PAI-1. Induction of PAI-1 by exposure of lung epithelial cells to uPA is a newly recognized pathway by which PAI-1 could regulate local fibrinolysis and **urokinase**-dependent cellular responses in the setting of lung inflammation or neoplasia.

L15 ANSWER 12 OF 129 MEDLINE on STN

2003064520. PubMed ID: 12574820. Human/chicken **urokinase** chimeras demonstrate sequences outside the serine protease domain that dictate autoactivation. Aimes Ronald T; Regazzoni Karine; Quigley James P. (The

Scripps Research Institute, Department of Cell Biology, VB 1, La Jolla, California 92037, USA.) Thrombosis and haemostasis, (2003 Feb) 89 (2) 382-92. Journal code: 7608063. ISSN: 0340-6245. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB Mammalian **urokinase**-type plasminogen activator (uPA) is produced as a stable single polypeptide chain zymogen and requires a distinct proteolytic cleavage to become an active, two-chain enzyme. In contrast, chicken uPA, both native and recombinant, is found predominantly as a two-chain, active enzyme even in the absence of plasmin, a physiological activator. Here we show that the proclivity to autoactivate is not a unique property of the chicken uPA catalytic domain but requires sequences distinct from and independent of the serine protease domain. Human/chicken chimeric uPA molecules and point mutants were used to determine the structural requirements for uPA autoactivation versus zymogen stability. The **amino terminal fragment** of chicken uPA engineered onto the human uPA molecule can induce the autoactivation of the human uPA. In fact, the first twenty residues of the chicken uPA are necessary and sufficient to induce the autoactivation of chicken and human uPA. These results indicate that sequence motifs, distal to the active site, control the substrate specificity and catalytic efficiency of uPA activity in autolytic activation.

L15 ANSWER 13 OF 129 MEDLINE on STN

2002718768. PubMed ID: 12479856. The diphtheria toxin/**urokinase** fusion protein (DTAT) is selectively toxic to CD87 expressing leukemic cells. Ramage Jason G; Vallera Daniel A; Black Jennifer H; Aplan Peter D; Kees Ursula R; Frankel Arthur E. (Department of Cancer Biology, Wake Forest University School of Medicine, Medical Center Boulevard, 27157, Winston-Salem, NC, USA.) Leukemia research, (2003 Jan) 27 (1) 79-84. Journal code: 7706787. ISSN: 0145-2126. Pub. country: England: United Kingdom. Language: English.

AB Diphtheria fusion proteins are a novel class of agents for the treatment of chemotherapy resistant acute myelogenous leukemia (AML). We prepared diphtheria toxin/**urokinase** fusion protein (DTAT) composed of the **amino terminal fragment** of the **urokinase**-type plasminogen activator (uPA) fused to the catalytic and translocation domains of diphtheria toxin (DT) and assessed its activity on leukemic cell lines. The number of uPA receptors (uPAR or CD87) was measured using a phycoerythrin conjugated monoclonal antibody to CD87 and flow cytometry. Seven of 23 cell lines (30%) showed CD87 expression (> or =5000 receptors/cell). DTAT cytotoxicity (IC(50)< or =30pM) was observed in all seven of these samples and none of the 16 samples with low or absent CD87 expression. There was a significant correlation between DTAT sensitivity and CD87 density (P=0.0007). These results show that specific CD87 binding is one factor important in the sensitivity of patient's leukemic blasts to DTAT and demonstrate for the first time that the CD87/uPAR can be used as a target for fusion protein therapy of AML.

L15 ANSWER 14 OF 129 MEDLINE on STN

2002673180. PubMed ID: 12433840. Gene transfer of the **urokinase**-type plasminogen activator receptor-targeted matrix metalloproteinase inhibitor TIMP-1.**ATF** suppresses neointima formation more efficiently than tissue inhibitor of metalloproteinase-1. Lamfers M L M; Grimbergen J M; Aalders M C; Havenga M J; de Vries M R; Huisman L G M; van Hinsbergh V W M; Quax P H A. (Gaubius Laboratory TNO-PG, Leiden, The Netherlands.) Circulation research, (2002 Nov 15) 91 (10) 945-52. Journal code: 0047103. ISSN: 1524-4571. Pub. country: United States. Language: English.

AB Proteases of the plasminogen activator (PA) and matrix metalloproteinase (MMP) system play an important role in smooth muscle cell (SMC) migration and neointima formation after vascular injury. Inhibition of either PAs or MMPs has previously been shown to result in decreased neointima formation in vivo. To inhibit both protease systems simultaneously, a novel hybrid protein, TIMP-1.**ATF**, was constructed consisting of the tissue inhibitor of metalloproteinase-1 (TIMP-1) domain, as MMP inhibitor, linked to the receptor-binding **amino terminal fragment** (**ATF**) of **urokinase**. By binding to the u-PA receptor this protein will not only

anchor the TIMP-1 moiety directly to the cell surface, it will also prevent the local activation of plasminogen by blocking the binding of **urokinase**-type plasminogen activator (u-PA) to its receptor. Adenoviral expression of TIMP-1.**ATF** was used to inhibit SMC migration and neointima formation in human saphenous vein segments in vitro. SMC migration was inhibited by 65% in Ad.TIMP-1.**ATF**-infected cells. Infection with adenoviral vectors encoding the individual domains, Ad.TIMP-1 and Ad.**ATF**, reduced migration by 32% and 52%, respectively. Neointima formation in saphenous vein organ cultures infected with Ad.TIMP-1.**ATF** was inhibited by 72% compared with 42% reduction after Ad.TIMP-1 infection and 34% after Ad.**ATF** infection. These data show that binding of TIMP-1.**ATF** hybrid protein to the u-PA receptor at the cell surface strongly enhances the inhibitory effect of TIMP-1 on neointima formation in human saphenous vein cultures.

L15 ANSWER 15 OF 129 MEDLINE on STN

2002672115. PubMed ID: 12408709. Synthesis, solution structure, and biological evaluation of **urokinase** type plasminogen activator (uPA)-derived receptor binding domain mimetics. Schmiedeberg Niko; Schmitt Manfred; Rolz Christian; Truffault Vincent; Sukopp Martin; Burgle Markus; Wilhelm Olaf G; Schmalix Wolfgang; Magdolen Viktor; Kessler Horst. (Institut fur Organische Chemie und Biochemie, Technische Universitat Munchen, Lichtenbergstrasse 4, D-85747 Garching, Germany.) Journal of medicinal chemistry, (2002 Nov 7) 45 (23) 4984-94. Journal code: 9716531. ISSN: 0022-2623. Pub. country: United States. Language: English.

AB Tumor cell migration and metastasis in cancer are facilitated by interaction of the serine protease **urokinase** type plasminogen activator (uPA) with its receptor uPAR (CD 87). Overexpression of uPA and uPAR in cancer tissues is associated with a high incidence of disease recurrence and early death. In agreement with these findings, disruption of the protein-protein interaction between uPAR present on tumor cells and its ligand uPA evolved as an attractive intervention strategy to impair tumor growth and metastasis. For this, the uPAR antagonist cyclo[19,31][D-Cys(19)]-uPA(19)(-)(31) was optimized to efficiently interrupt binding of uPA to cellular uPAR. First, the disulfide bridge of this lead compound was shifted and then the modified peptide was shortened from the amino and carboxy terminus to generate cyclo[21,29][Cys(21,29)]-uPA(21)(-)(30). Next, cyclo[21,29][D-Cys(21)Cys(29)]-uPA(21)(-)(30) was yielded by changing the chirality of Cys(21) to D-Cys(21). For analysis of uPAR binding activity, we employed competitive flow cytofluorometric receptor binding assays, using FITC-uPA as the ligand and U937 promyeloid leukemia cells as the cellular source of uPAR. As demonstrated for cyclo[21,29][D-Cys(21)Cys(29)]-uPA(21)(-)(30), the achieved peptide modifications maintained receptor binding activity (IC(50) = 0.04 microM), which is close in order to that of the parent protein ligand, uPA (IC(50) = 0.01 microM). A detailed NMR analysis with restrained and free molecular dynamics calculations in explicit H(2)O exhibits a well-defined structure with characteristic features such as an omega-loop with two betaI-turns about Lys(3), Tyr(4), Ser(6), and Asn(7). Hydrophobic clustering of the side chains of Tyr(4), Phe(5), Ile(8), and Trp(10) is observed. Side chain mobility is analyzed with time-dependent distance restraints. The NMR structure of cyclo[21,29][D-Cys(21)Cys(29)]-uPA(21)(-)(30) is very similar to the previously reported structure of the **amino terminal fragment** of uPA. Systematic point mutations led to cyclo[21,29][D-Cys(21)Nle(23)Cys(29)]-uPA(21)(-)(30), which still binds to uPAR but is resistant to proteolytic cleavage, e.g., by the tumor-associated serine proteases uPA and plasmin, and is stable in blood serum or plasma. In conclusion, small cyclic peptides were created, which mimic the structure and activity of the binding epitope of uPA to uPAR and which may serve as novel therapeutic agents in cancer metastasis.

L15 ANSWER 16 OF 129 MEDLINE on STN

2002660195. PubMed ID: 12420219. Modulation of invasive properties of human glioblastoma cells stably expressing **amino-terminal fragment** of **urokinase**-type plasminogen activator. Mohanam Sanjeeva; Chandrasekar Nirmala; Yanamandra Niranjan; Khawar Siddique; Mirza Faiz; Dinh Dzung H;

Olivero William C; Rao Jasti S. (Division of Cancer Biology, Department of Biomedical and Therapeutic Sciences, University of Illinois College of Medicine at Peoria, Peoria, Illinois 61656-1649, USA.) Oncogene, (2002 Nov 7) 21 (51) 7824-30. Journal code: 8711562. ISSN: 0950-9232. Pub. country: England: United Kingdom. Language: English.

AB The binding of **urokinase**-type plasminogen activator (uPA) to its receptor (uPAR) on the surface of tumor cells is involved in the activation of proteolytic cascades responsible for the invasiveness of those cells. The diffuse, extensive infiltration of glioblastomas into the surrounding normal brain tissue is believed to rely on modifications of the proteolysis of extracellular matrix components; blocking the interaction between uPA and uPAR might be a suitable approach for inhibiting glioma tumorigenesis. We assessed how expression of an **amino-terminal fragment** (**ATF**) of uPA that contains binding site to uPAR affects the invasiveness of SNB19 human glioblastoma cells. SNB19 cells were transfected with an expression plasmid (pcDNA3-**ATF**) containing a cDNA sequence of **ATF**-uPA. The resulting **ATF**-uPA-expressing clones showed markedly less cell adhesion, spreading, and clonogenicity than did control cells. Endogenous **ATF** expression also significantly decreased the invasive capacity of transfected glioblastoma cells in Matrigel and spheroid-rat brain cell aggregate models. **ATF**-uPA transfectants were also markedly less invasive than parental SNB19 cells after injection into the brains of nude mice, suggesting that competitive inhibition of the uPA-uPAR interaction on SNB19 cells by means of transfection with **ATF** cDNA could be a useful therapeutic strategy for inhibiting tumor progression.

L15 ANSWER 17 OF 129 MEDLINE on STN

2002643285. PubMed ID: 12182908. **Amino-terminal fragment** of **urokinase**-type plasminogen activator inhibits its plasminogen activation. Sun Ziyong; Zhang Pei-Xiang; Wang Ping; Gurewich Victor; Shen Hai-Yan; Liu Jian-Ning. (Institute of Molecular Medicine, Nanjing University, Nanjing 210093, PR China.) Thrombosis research, (2002 Apr 15) 106 (2) 105-11. Journal code: 0326377. ISSN: 0049-3848. Pub. country: United States. Language: English.

AB The **amino terminal fragment** (**ATF**, Ser(1)-Lys(135)) of **urokinase**-type plasminogen activator (uPA) containing an epidermal growth factor-like (EGF) and kringle domain is critically involved in some important functions of uPA, such as receptor binding and chemotactic activity. In this report, the effect of **ATF** on single-chain uPA (sc-uPA) induced plasminogen activation was investigated. It was shown that sc-uPA-induced activation of Glu-plasminogen or Lys-plasminogen was significantly inhibited in the presence of **ATF**. In addition, sc-uPA activation to two-chain uPA (tc-uPA) by Lys-plasmin and plasminogen activation to plasmin by tc-uPA were both found to be inhibited by **ATF**. The inhibition of these activations was significantly attenuated but not diminished when **ATF** was pretreated with immobilized carboxypeptidase B (CPB), indicating that the C-terminal Lys(135) as well as internal Lys/Arg residue binding was involved in the mechanism. Kinetic analysis showed that sc-uPA activation by Lys-plasmin competitively inhibited by **ATF** and CPB pretreated **ATF** (CPB-**ATF**) with an inhibitory constant (K(i)) of 3.8+/-0.31 and 12.4 +/- 1.8 microM, respectively. In contrast to sc-uPA-induced Glu- or Lys-plasminogen activation, sc-uPA-induced mini-plasminogen activation, sc-uPA activation by mini-plasmin and mini-plasminogen activation by tc-uPA were not affected by **ATF**. These findings suggested that the inhibitory effects of **ATF** on sc-uPA activation by Lys-plasmin and Glu- or Lys-plasminogen activation by tc-uPA were related to the binding of **ATF** (by its C-terminal Lys(135) and internal Lys/Arg residue) with the kringle 1-4 of plasmin and plasminogen, respectively.

L15 ANSWER 18 OF 129 MEDLINE on STN

2002613342. PubMed ID: 12369939. Inhibitors of the protease domain of **urokinase**-type plasminogen activator. Rockway T W; Nienaber V; Giranda V L. (Department of Cancer Research, Global Pharmaceutical Research and

Development, Abbott Laboratories, Abbott Park, IL 60064-6217, USA.. todd.w.rockway@abbott.com) . Current pharmaceutical design, (2002) 8 (28) 2541-58. Ref: 95. Journal code: 9602487. ISSN: 1381-6128. Pub. country: Netherlands. Language: English.

AB Human **urokinase**-type plasminogen activator (uPA or uPA) has been implicated in the regulation and control of basement membrane and interstitial protein degradation. Since **Urokinase** plays a role in tissue remodeling, it may be responsible, in part, for the disease progression of cancer. Inhibitors of **urokinase** may then be useful in the treatment of cancer by retarding tumor growth and metastasis. **Urokinase** is a multidomain protein, two regions of the protein are most responsible for the observed proteolytic activity in cancer disease and progression. The N-terminal domain or **ATF** binds to a **Urokinase** receptor (uPAR) on the cell surface and the C-terminal serine protease domain, then, activates plasminogen to plasmin, beginning a cascade of events leading to the progression of cancer. Investigations of **urokinase** inhibition has been an area of ongoing research for the past 3 decades. It began with the discovery of small natural and unnatural amino acid derivatives or peptide analogs which exhibited weak inhibition of uPA. The last decade has seen the generation of several classes of potent and selective **Urokinase** inhibitor directed to the serine protease domain of the protein which have shown potential anti-cancer effects. The availability of structural information of enzyme-inhibitor complexes either by nuclear magnetic spectroscopy (NMR) or crystallography has allowed a detailed analysis of inhibitor protein interactions that contribute to observed inhibitor potency. Structural studies of specific inhibitor-uPA complexes will be discussed as well as the contributions of specific inhibitor protein interactions that are important for overall inhibitor potency. These data were used to discover a class of **urokinase** inhibitor based on the 2-Naphthamidine template that exhibits potent **urokinase** inhibition and excellent selectivity for **urokinase** over similar trypsin family serine proteases.

L15 ANSWER 19 OF 129 MEDLINE on STN

2002463708. PubMed ID: 12222798. Expression of **urokinase** plasminogen activator receptor in resting and activated bovine neutrophils. Politis Ioannis; Zavizjon Boris; Cheli Federica; Baldi Antonella. (Department of Animal Production, Agricultural University of Athens, Greece.) Journal of dairy research, (2002 May) 69 (2) 195-204. Journal code: 2985125R. ISSN: 0022-0299. Pub. country: England: United Kingdom. Language: English.

AB Changes in **urokinase**-plasminogen activator (u-PA) and u-PA receptor (u-PAR) expression at the protein and mRNA level in resting neutrophils and in neutrophils activated by phorbol myristate acetate (PMA) were examined. Low amounts of u-PA were found intracellularly or membrane-bound in resting neutrophils. However, incubation of resting neutrophils with purified exogenous u-PA (10 IU/ml) revealed extensive binding of u-PA to cell membranes. Excess **amino-terminal fragment** of the u-PA molecule, a proteolytically inactive fragment of u-PA (amino acids 1-135) blocked binding of exogenous u-PA to the cell membrane. These results, collectively, indicate that the binding of u-PA is specific and that resting neutrophils have unoccupied u-PA receptors on their cell membrane. Addition of PMA led to an increase ($P < 0.01$) in total cell-associated, membrane-bound u-PA activity and u-PA mRNA expression by bovine neutrophils. In contrast. PMA increased u-PAR mRNA levels but this was accompanied by a decrease (2.5-fold; $P < 0.01$) in free, unoccupied u-PA binding sites. No significant effects on total cell-associated or membrane-bound u-PA were found when neutrophils were treated with 4-phorbol 12,13 didecanoate, a phorbol ester that does not activate protein kinase C (PKC). Furthermore, addition of 1-(5-isoquinolinesylphonyl)-2-methlylpiperazine dihydrochloride (H-7), a potent PKC inhibitor, blocked the effect of PMA on total cell-associated u-PA activity. Thus, PKC plays a role in the modulation of u-PA and u-PAR by PMA in bovine neutrophils.

L15 ANSWER 20 OF 129 MEDLINE on STN

2002426205. PubMed ID: 12183060. **Urokinase** is required for the formation

of mactinin, an alpha actinin fragment that promotes monocyte/macrophage maturation. Luikart Sharon; Masri Mohammed; Wahl Dan; Hinkel Tim; Beck James M; Gyetko Margaret R; Gupta Pankaj; Oegema Theodore. (Veterans Affairs Medical Center, Minneapolis, MN, USA.. sharon.luikart@med.va.gov) . Biochimica et biophysica acta, (2002 Aug 19) 1591 (1-3) 99-107. Journal code: 0217513. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.

AB We have previously shown that lysates from HL-60 myeloid leukemia cells or from peripheral blood monocytes are able to degrade alpha-actinin to form a 31-kDa **amino-terminal fragment** with monocyte/macrophage maturation promoting activity. In contrast, intact alpha-actinin, which is a 100-kDa actin-binding protein, has no differentiating activity. The aim of this study was to investigate the enzyme responsible for the degradation of alpha-actinin to form this fragment, named mactinin. The ability of cell lysates to degrade [125I]alpha-actinin in the presence of various enzyme inhibitors, including inhibitors of metalloproteinases, cysteine proteinases, and serine proteases, was measured. Phenylmethylsulfonyl fluoride (PMSF) was the only inhibitor able to prevent formation of mactinin by cell lysate degradation of alpha-actinin, suggesting that a serine protease is responsible for the digestion. Of the various serine proteases tested (thrombin, plasmin, and **urokinase**), only **urokinase** was able to produce a 31-kDa band. The **urokinase**-generated 31-kDa band promoted maturation in HL-60 cells. Amiloride, a specific inhibitor of **urokinase**, inhibited production of the 31-kDa alpha-actinin fragment by HL-60 cell lysates. For in vivo tests, inflammatory fluid (from bronchoalvelolar lavage) was collected from uPA (**urokinase**) knockout mice and their wild-type counterparts after intratracheal challenge with Pneumocystis carinii. Although most (6 of 8) wild-type mice had mactinin in their inflammatory fluid samples, none (0 of 8) of the uPA knockout mice had mactinin present ($P<0.01$). These results demonstrate that **urokinase** is necessary and sufficient for the formation of the monocyte/macrophage maturation promoting fragment, mactinin, in vitro and in vivo. These findings support the role of **urokinase** in the regulation of monocyte/macrophage functions, such as that occurring in inflammatory reactions.

L15 ANSWER 21 OF 129 MEDLINE on STN

2002398143. PubMed ID: 12147175. Adenoviral gene transfer of angiostatic **ATF**-BPTI inhibits tumour growth. Lefesvre Pierre; Attema Joline; van Bekkum Dirk. (Crucell BV, Leiden, The Netherland.. p.lefesvre@crucell.com) . BMC cancer [electronic resource], (2002 Jul 29) 2 (1) 17. Journal code: 100967800. ISSN: 1471-2407. Pub. country: England: United Kingdom. Language: English.

AB BACKGROUND: The outgrowth of new vessels--angiogenesis--in the tumour mass is considered to be a limiting factor of tumour growth. To inhibit the matrix lysis that is part of the tumour angiogenesis, we employed the chimeric protein mhATF-BPTI, composed of the receptor binding part of the **urokinase** (**ATF**) linked to an inhibitor of plasmin (BPTI). METHODS: For delivery, recombinant adenovirus encoding the transgene of interest was injected intravenously or locally into the tumour. The anti tumour effect of this compound was compared to that of human endostatin and of mhATF alone in two different rat bronchial carcinomas growing either as subcutaneous implants or as metastases. RESULTS: Significant inhibition of the tumour growth and decrease of the number of lung metastasis was achieved when the concentration of mhATF-BPTI at the tumour site was above 400 of ng/g tissue. This concentration could be achieved via production by the liver, only if permissive to the recombinant adenovirus. When the tumour cells could be transduced, local delivery of the vector was enough to obtain a response. In the case of metastasis, the capacity of the lung tissue to concentrate the encoded protein was essential to reach the required therapeutic levels. Further, endostatin or mhATF could not reproduce the effects of mhATF-BPTI, at similar concentrations (mhATF) and even at 10-fold higher concentration (endostatin). CONCLUSION: The **ATF**-BPTI was shown to inhibit tumour growth of different rat lung tumours when critical concentration was reached. In these tumour models, endostatin or **ATF** induce almost no tumour response.

L15 ANSWER 22 OF 129 MEDLINE on STN
2002396326. PubMed ID: 12034711. **Urokinase** regulates vitronectin binding by controlling **urokinase** receptor oligomerization. Sidenius Nicolai; Andolfo Annapaola; Fesce Riccardo; Blasi Francesco. (Molecular Genetics Unit, DIBIT, Universita Vita-Salute San Raffaele, 20132 Milan, Italy.. sidenius.nicolai@hsr.it) . Journal of biological chemistry, (2002 Aug 2) 277 (31) 27982-90. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Adhesion of monocytes to the extracellular matrix is mediated by a direct high affinity interaction between cell-surface **urokinase**-type plasminogen activator (uPA) receptor (uPAR) and the extracellular matrix protein vitronectin. We demonstrate a tight connection between uPA-regulated uPAR oligomerization and high affinity binding to immobilized vitronectin. We find that binding of soluble uPAR (suPAR) to immobilized vitronectin is strictly ligand-dependent with a linear relationship between the observed binding and the concentration of ligand added. Nevertheless, a comparison of experimentally obtained binding curves to those generated using a simple equilibrium model suggests that the high affinity vitronectin-binding pro-uPA.suPAR complex contains two molecules of suPAR. In co-immunoprecipitation experiments, using different epitope-tagged suPAR molecules, suPAR/suPAR co-immunoprecipitation displayed a similar uPA dose dependence as that observed for vitronectin binding, demonstrating that the high affinity vitronectin-binding complex indeed contains oligomeric suPAR. Structurally, the kringle domain of uPA was found to be critical for the formation of the vitronectin-binding competent complex because the **amino-terminal fragment**, but not the growth factor-like domain, behaved as a full-length uPA. Our data represent the first demonstration of functional, ligand-induced uPAR oligomerization having extensive implications for glycosylphosphatidylinositol-anchored receptors in general, and for the biology of the uPA/uPAR system in particular.

L15. ANSWER 23 OF 129 MEDLINE on STN
2002369602. PubMed ID: 12114193. **Urokinase** induces its own expression in Beas2B lung epithelial cells. Shetty Sreerama; Pendurthi Usha R; Halady Prathap Kumar Shetty; Azghani Ali O; Idell Steven. (Department of Medical Specialties, The University of Texas Health Center at Tyler, Tyler, Texas 75708, USA.. sreerama.shetty@uthct.edu) . American journal of physiology. Lung cellular and molecular physiology, (2002 Aug) 283 (2) L319-28. Journal code: 100901229. ISSN: 1040-0605. Pub. country: United States. Language: English.

AB The **urokinase**-type plasminogen activator (uPA) interacts with its receptor (uPAR) to promote local proteolysis as well as cellular proliferation and migration. These functions contribute to the pathogenesis of lung inflammation and remodeling as well as the growth and invasiveness of lung neoplasms. In this study, we sought to determine if uPA alters its own expression in lung epithelial cells. Using immunoprecipitation and Western and Northern blotting techniques, we found that uPA treatment enhanced uPA expression in Beas2B lung epithelial cells in a time- and concentration-dependent manner. The induction of uPA expression is mediated through its cell surface receptor uPAR and does not require uPA enzymatic activity. The **amino-terminal fragment** of uPA, lacking the catalytic domain, is sufficient to induce uPA expression. The serine protease plasmin and the protease inhibitor aprotinin failed to alter uPA-mediated uPA expression, whereas alpha-thrombin potentiated the response. Pretreatment of Beas2B cells with a tyrosine kinase inhibitor, herbimycin, suggests that activation of tyrosine kinase(s) is involved in the uPA-mediated uPA expression. Induction of uPA expression by exposure of lung-derived epithelial cells to uPA is a newly defined pathway by which this protease could influence expression of local fibrinolytic activity and other uPA-dependent cellular responses germane to lung inflammation or neoplasia.

L15 ANSWER 24 OF 129 MEDLINE on STN
2002198123. PubMed ID: 11930939. uPA-silica-Particles (SP-uPA): a novel

analytical system to investigate uPA-uPAR interaction and to test synthetic uPAR antagonists as potential cancer therapeutics. Guthaus Elke; Burgle Markus; Schmiedeberg Niko; Hocke Stefan; Eickler Alexandra; Kramer Michael D; Sweep C G J Fred; Magdolen Viktor; Kessler Horst; Schmitt Manfred. (Klinische Forschergruppe, Frauenklinik der TU Munchen, Germany.) Biological chemistry, (2002 Jan) 383 (1) 207-16. Journal code: 9700112. ISSN: 1431-6730. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB The **urokinase**-type plasminogen activation system, including the serine protease uPA (**urokinase**-type plasminogen activator) and its cell surface receptor (uPAR, CD87), are important key molecules in tumor invasion and metastasis. Besides its proteolytic function, binding of uPA to uPAR on tumor cells exerts various cell responses such as migration, adhesion, proliferation, and differentiation. Hence, the uPA/uPAR system is a potential target for tumor therapy. We have designed a new generation of uPA-derived synthetic cyclic peptides suited to interfere with the binding of uPA to uPAR and present a new technology involving micro silica particles coated with uPA (SP-uPA) and reacting with recombinant soluble uPAR (suPAR), to rapidly assess the antagonistic potential of uPA-peptides by flow cytofluorometry (FACS). For this, we used silica particles of 10 microm in diameter to which HMW-uPA is coupled using the EDC/NHS method. Soluble, recombinant suPAR was added and the interaction of SP-uPA with suPAR verified by reaction with monoclonal antibody HD13.1 directed to uPAR, followed by a cyan dye (cy5)-labeled antibody directed against mouse IgG. Thereby it was possible to test naturally occurring ligands of uPAR (HMW-uPA, **ATF**) as well as highly effective, synthetic cyclic uPA-derived peptides (cyclo21,29[D-Cys21Cys29]-UPA21-30, cyclo21,29[D-Cys21Nle28Cys29]-uPA21-30, cyclo21,29[D-Cys(21)2-Nal24Cys29]-uPA21-30, and cyclo21,29[D-Cys21Orn23Thi24Thi25Cys29]-uPA21-30. The results obtained with the noncellular SP-uPA/uPAR system are highly comparable to those obtained with a cellular system involving FITC-uPA and the promyeloid cell line U937 as the source of uPAR.

L15 ANSWER 25 OF 129 MEDLINE on STN

2002123993. PubMed ID: 11859422. Adenovirus-mediated gene transfer of **urokinase** plasminogen inhibitor inhibits angiogenesis in experimental arthritis. Apparailly F; Bouquet C; Millet V; Noel D; Jacquet C; Opolon P; Perricaudet M; Sany J; Yeh P; Jorgensen C. (INSERM U475, Montpellier, France.) Gene therapy, (2002 Feb) 9 (3) 192-200. Journal code: 9421525. ISSN: 0969-7128. Pub. country: England: United Kingdom. Language: English.

AB Plasmin is essential for metalloproteases activation, endothelial cell migration and degradation of the extracellular matrix. The process is common to neoangiogenesis pannus formation and cartilage degradation within arthritic joints. Since 80% of synovial cells express **urokinase** plasminogen activator receptor (uPAR), we investigated the inhibition of plasmin activation in a collagen-induced arthritis (CIA) mice model, by expressing a uPA/uPAR antagonist molecule (**ATF**) fused to human serum albumin (HSA) to extend its serum half-life. Overexpression was obtained with an adenoviral vector expressing the chimeric murine **ATF**-HSA. We showed that the genetic coupling did not significantly reduce the ability of the **ATF** moiety to interact with its receptor uPAR. The chimeric protein was detectable in the sera of injected mice 7 days following Ad-mATF-HSA injection, then decreased in parallel with the anti-HSA titer increase. Systemic Ad-mATF-HSA injection performed on day 25 following CIA induction decreased the incidence of arthritis and the severity of the disease. Moreover, synovial angiogenesis in arthritic paws was decreased after Ad-mATF-HSA gene transfer, as assessed by smooth muscle actin immunostaining. The preventive effect observed on arthritis was related to the decrease in angiogenesis, rather than inhibition of extracellular matrix degradation.

L15 ANSWER 26 OF 129 MEDLINE on STN

2002057483. PubMed ID: 11783013. Experimental study of anti-metastasis effect of **urokinase amino-terminal fragment** gene on human breast cancer cells. Zhu F; Xing G; He F. (Beijing Institute of Radiation Medicine, Beijing 100850, China.) Zhonghua zhong liu za zhi [Chinese

Journal of Oncology], (2001 Mar) 23 (2) 113-7. Journal code: 7910001. ISSN: 0253-3766. Pub. country: China. Language: Chinese.

AB OBJECTIVE: To explore the suppressive effects of **urokinase amino-terminal fragment** (**ATF**) gene on metastatic potential of human breast cancer cell line MCF-7. METHODS: A pcDNA3-**ATF** plasmid containing **ATF** cDNA under CMV promotor/enhancer control was constructed and transfected into MCF-7 cells by lipofectin. The expression of of uPA/uPAR and **ATF** in MCF-7 cells were analyzed by RT-PCR and Western blot. The effect of **ATF** expression on invasiveness in vitro, tumorigenesis and metastasis in vivo of MCF-7 cell was investigated. RESULTS: MCF-7 cells displayed an overexpression of uPA/uPAR. Expression of **ATF** was detected after **ATF** gene-transfection. The invasive capacity of **ATF** gene-transfected MCF-7 cells was decreased significantly. Although the tumorigenesis was not affected, the in vivo metastasis of **ATF** gene-transfected MCF-7 cells was remarkably inhibited. CONCLUSION: Suppression of invasiveness and metastasis of **ATF**-transfected MCF-7 cells is perhaps due to a competitive inhibition of interaction with endogenous uPA/uPAR.

L15 ANSWER 27 OF 129 MEDLINE on STN

2001565487. PubMed ID: 11672584. **Urokinase**-type plasminogen activator up-regulates its own expression by endothelial cells and monocytes via the u-PAR pathway. Li C; Zhang J; Jiang Y; Gurewich V; Chen Y; Liu J N. (Institute of Molecular Medicine, Nanjing University, Nanjing 10008, China.) Thrombosis research, (2001 Aug 1) 103 (3) 221-32. Journal code: 0326377. ISSN: 0049-3848. Pub. country: United States. Language: English.

AB Signal transduction by **urokinase**-type plasminogen activator (u-PA) bound to its cell receptor has been well established. In the present study, we found, for the first time to our knowledge, that u-PA promotes its own synthesis by endothelial cells and monocytes. This phenomenon was characterized and shown to involve the u-PA receptor (u-PAR) pathway. The finding may be of general importance, since most cells that express u-PAR also produce u-PA. Human umbilical vein endothelial cells (HUVECs), U937 monocytes, and human peripheral blood monocytes (PFMCs) were incubated with diisopropylfluorophosphate (DFP)-pretreated u-PA, the **amino-terminal fragment** (**ATF**) of u-PA, or the kringle domain. A threefold up-regulation of u-PA secretion and synthesis by u-PA or **ATF** was found. The predominant effect was expressed in HUVECs, in which u-PA mRNA was also up-regulated. The u-PA kringle domain had no effect on u-PA synthesis, leading to the conclusion that the EGF domain was responsible. This was also consistent with the additional finding that the u-PAR, to which the EGF domain binds, was necessary for the up-regulation. The results indicate that u-PA up-regulates itself via its EGF domain and u-PAR. The possibilities that the results were related to displacement of receptor-bound u-PA or the blocking of u-PA incorporation into the cells were excluded. A modest up-regulation of u-PAR was also associated with this phenomenon.

L15 ANSWER 28 OF 129 MEDLINE on STN

2001545564. PubMed ID: 11592401. Cyclo19,31[D-Cys19]-uPA19-31 is a potent competitive antagonist of the interaction of **urokinase**-type plasminogen activator with its receptor (CD87). Magdolen V; Burgle M; de Prada N A; Schmiedeberg N; Riemer C; Schroeck F; Kellermann J; Degitz K; Wilhelm O G; Schmitt M; Kessler H. (Frauenklinik der Technischen Universitat Munchen, Germany.) Biological chemistry, (2001 Aug) 382 (8) 1197-205. Journal code: 9700112. ISSN: 1431-6730. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB **Urokinase**-type plasminogen activator (uPA) represents a central molecule in pericellular proteolysis and is implicated in a variety of physiological and pathophysiological processes such as tissue remodelling, wound healing, tumor invasion, and metastasis. uPA binds with high affinity to a specific cell surface receptor, uPAR (CD87), via a well defined sequence within the N-terminal region of uPA (uPA19-31). This interaction directs the proteolytic activity of uPA to the cell surface which represents an important step in tumor cell proliferation, invasion, and metastasis. Due to its fundamental role in these processes, the

uPA/uPAR system has emerged as a novel target for tumor therapy. Previously, we have identified a synthetic, cyclic, uPA-derived peptide, cyclo19,31uPA19-31, as a lead structure for the development of low molecular weight uPA-analogues, capable of blocking uPA/uPAR-interaction [Burgle et al., Biol. Chem. 378 (1997), 231-237]. We now searched for peptide variants of cyclo19,31uPA19-31 with elevated affinities for uPAR binding. Among other tasks, we performed a systematic D-amino acid scan of uPA19-31, in which each of the 13 L-amino acids was individually substituted by the corresponding D-amino acid. This led to the identification of cyclo19,31[D-Cys19]-uPA19-31 as a potent inhibitor of uPA/uPAR-interaction, displaying only a 20 to 40-fold lower binding capacity as compared to the naturally occurring uPAR-ligands uPA and its **amino-terminal fragment**. Cyclo19,31[D-Cys19]-uPA19-31 not only blocks binding of uPA to uPAR but is also capable of efficiently displacing uPAR-bound uPA from the cell surface and to inhibit uPA-mediated, tumor cell-associated plasminogen activation and fibrin degradation. Thus, cyclo19,31[D-Cys19]-uPA19-31 represents a promising therapeutic agent to significantly affect the tumor-associated uPA/uPAR-system.

L15 ANSWER 29 OF 129 MEDLINE on STN

2001346125. PubMed ID: 11410166. cDNA transfection of **amino-terminal fragment** of **urokinase** efficiently inhibits cancer cell invasion and metastasis. Zhu F; Jia S; Xing G; Gao L; Zhang L; He F. (Beijing Institute of Radiation Medicine, Beijing, China.) DNA and cell biology, (2001 May) 20 (5) 297-305. Journal code: 9004522. ISSN: 1044-5498. Pub. country: United States. Language: English.

AB Focusing of **urokinase**-type plasminogen activator (uPA) to the cell surface via binding to its specific receptor (uPAR, CD87) is critical for tumor invasion and metastasis. Consequently, the inhibition of uPA-uPAR interaction on the cell surface might be a promising anti-invasion and anti-metastasis strategy. We examined the effects of cDNA transfection of the human uPA **amino-terminal fragment** (**ATF**) on invasion and metastasis of cancer cells. First, a highly metastatic human lung giant-cell carcinoma cell line (PG), used as the target cell for evaluation of this effect, was demonstrated to express both uPA and uPAR. Then, **ATF**, which contains an intact uPAR binding site but is catalytically inactive, was designed as an antagonist of uPA-uPAR interaction and was transfected into PG cells. [(3)H]-Thymidine incorporation and cell growth curves indicated that expressed **ATF** did not affect the proliferation of transfected cells. However, analysis by scanning electron microscopy revealed that **ATF** changed the host cells from the typical invasive phenotype to a noninvasive one. Correspondingly, the modified Boyden chamber test in vitro showed that **ATF** expression significantly decreased the invasive capacity of transfected cells. Furthermore, in the spontaneous metastasis model, it was confirmed in vivo that expressed **ATF** remarkably inhibited lung metastasis of implanted **ATF**-transfected PG cells. In summary, autocrine **ATF** could act as an antagonist of uPA-uPAR interaction, and **ATF** cDNA transfection could efficiently inhibit the invasion and metastasis of the cancer cells. Inhibition of uPA-uPAR interaction on the cell surface might be a promising anti-invasion and anti-metastasis strategy.

L15 ANSWER 30 OF 129 MEDLINE on STN

2001327647. PubMed ID: 11394884. **Amino-terminal fragment** of **urokinase**-type plasminogen activator inhibits HIV-1 replication. Wada M; Wada N A; Shirono H; Taniguchi K; Tsuchie H; Koga J. (Laboratories for Bioengineering and Research, JCR Pharmaceuticals Company, Ltd., 2-2-10 Murotani, Nishi-ku, Kobe, 651-2241, Japan.. wada-m@jcrpharm.co.jp) . Biochemical and biophysical research communications, (2001 Jun 8) 284 (2) 346-51. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB CD8+ T lymphocytes have been shown to produce unidentified soluble factors active in suppressing HIV-1 replication. In this study, we purified an HIV-1 suppressing activity from the culture supernatant of an immortalized CD8+ T cell clone, derived from an HIV-1 infected long-term nonprogressor,

and identified this activity as the **amino terminal fragment** (**ATF**) of **urokinase**-type plasminogen activator (uPA). **ATF** is catalytically inactive, but suppresses the release of viral particles from the HIV-1 infected cell lines via binding to its receptor CD87. In contrast, cell proliferation and the secretion of an HIV-1 LTR driven reporter gene product were not affected by **ATF**. These findings suggest that **ATF** may inhibit the assembly and budding of HIV-1, which provides a novel therapeutic strategy for AIDS.

L15 ANSWER 31 OF 129 MEDLINE on STN
2001324898. PubMed ID: 11319620. In vivo suppression of restenosis in balloon-injured rat carotid artery by adenovirus-mediated gene transfer of the cell surface-directed plasmin inhibitor **ATF**.BPTI. Lamfers M L; Lardenoye J H; de Vries M R; Aalders M C; Engelse M A; Grimbergen J M; van Hinsbergh V W; Quax P H. (Gaubius Laboratory TNO-PG, 2301 CE Leiden, The Netherlands.) Gene therapy, (2001 Apr) 8 (7) 534-41. Journal code: 9421525. ISSN: 0969-7128. Pub. country: England: United Kingdom. Language: English.
AB Injury-induced neointimal development results from migration and proliferation of vascular smooth muscle cells (SMC). Cell migration requires controlled proteolytic degradation of extracellular matrix surrounding the cell. Plasmin is a major contributor to this process by degrading various matrix proteins directly, or indirectly by activating matrix metalloproteinases. This makes it an attractive target for inhibition by gene transfer. An adenoviral vector, Ad.**ATF**.BPTI, was constructed encoding a hybrid protein, which consists of the aminoterminal fragment (**ATF**) of **urokinase**-type plasminogen activator (u-PA) linked to bovine pancreas trypsin inhibitor (BPTI), a potent inhibitor of plasmin. This hybrid protein binds to the u-PA receptor, thereby inhibiting plasmin activity at the cell surface, and was found to be a potent inhibitor of cell migration in vitro. Local infection with Ad.**ATF**.BPTI of balloon-injured rat carotid artery resulted in detectable expression of **ATF**.BPTI mRNA and protein in the vessel wall. Morphometric analysis of arterial cross-sections revealed that delivery of Ad.**ATF**.BPTI to the carotid artery wall at the time of balloon injury inhibited neointima formation by 53% ($P < 0.01$) at 14 days and 19% (P = NS) at 28 days after injury when compared with control vector-infected arteries. Intima/media ratios were decreased by 60% ($P < 0.01$) and 35% ($P < 0.05$) at 14 and 28 days, respectively, when compared with control vector-infected arteries. Furthermore, a small but significant increase in medial area was found in the Ad.**ATF**.BPTI-treated arteries at 28 days ($P < 0.05$). These results show that local infection of the vessel wall with Ad.**ATF**.BPTI reduces neointima formation, presumably by inhibiting SMC migration, thereby offering a novel therapeutic approach to inhibiting neointima development.

L15 ANSWER 32 OF 129 MEDLINE on STN
2001226300. PubMed ID: 11157723. Adenoviral expression of a **urokinase** receptor-targeted protease inhibitor inhibits neointima formation in murine and human blood vessels. Quax P H; Lamfers M L; Lardenoye J H; Grimbergen J M; de Vries M R; Slomp J; de Ruiter M C; Kockx M M; Verheijen J H; van Hinsbergh V W. (Gaubius Laboratory TNO-PG, Leiden, Netherlands.. pha.quax@pg.tno.nl) . Circulation, (2001 Jan 30) 103 (4) 562-9. Journal code: 0147763. ISSN: 1524-4539. Pub. country: United States. Language: English.
AB BACKGROUND: Smooth muscle cell migration, in addition to proliferation, contributes to a large extent to the neointima formed in humans after balloon angioplasty or bypass surgery. Plasminogen activator/plasmin-mediated proteolysis is an important mediator of this smooth muscle cell migration. Here, we report the construction of a novel hybrid protein designed to inhibit the activity of cell surface-bound plasmin, which cannot be inhibited by its natural inhibitors, such as alpha(2)-antiplasmin. This hybrid protein, consisting of the receptor-binding **amino-terminal fragment** of uPA (**ATF**), linked to the potent protease inhibitor bovine pancreas trypsin inhibitor (BPTI),

can inhibit plasmin activity at the cell surface. METHODS AND RESULTS: The effect of adenovirus-mediated **ATF**.BPTI expression on neointima formation was tested in human saphenous vein organ cultures. Infection of human saphenous vein segments with Ad.CMV.**ATF**.BPTI (5x10(9) pfu/mL) resulted in 87.5+/-3.8% (mean+/-SEM, n=10) inhibition of neointima formation after 5 weeks, whereas Ad.CMV.**ATF** or Ad.CMV.BPTI virus had only minimal or no effect on neointima formation. The efficacy of **ATF**.BPTI in vivo was demonstrated in a murine model for neointima formation. Neointima formation in the femoral artery of mice, induced by placement of a polyethylene cuff, was strongly inhibited (93.9+/-2%) after infection with Ad.CMV.mATF.BPTI, a variant of **ATF**.BPTI able to bind specifically to murine uPA receptor; Ad.CMV.mATF and Ad.CMV.BPTI had no significant effect. CONCLUSIONS: These data provide evidence that adenoviral transfer of a hybrid protein that binds selectively to the uPA receptor and inhibits plasmin activity directly on the cell surface is a powerful approach to inhibiting neointima formation and restenosis.

L15 ANSWER 33 OF 129 MEDLINE on STN

2001126569. PubMed ID: 11206835. Isolation and characterization of cell lines with reduced **urokinase** binding. Lau H K; Teitel J M; Kim M. (Department of Laboratory Medicine and Pathobiology, St. Michael's Hospital and University of Toronto, Ontario, Canada.. lauh@smh.toronto.on.ca) . Clinical & experimental metastasis, (2000) 18 (1) 29-36. Journal code: 8409970. ISSN: 0262-0898. Pub. country: Netherlands. Language: English.

AB Six cell lines have been generated from the human fibrosarcoma HT-1080 by mutagenesis. They were selected on the basis of reduced **urokinase** (uPA) binding on replicate polyester filters. Single cell clones were then isolated by limited dilution cloning. All cloned cells showed less uPA binding on filters, and as cell monolayers. These cell lines were able to bind only 10 to 65% as much uPA as the wild-type HT-1080 cells. Surface-bound uPA proteolytic activity and surface activation of plasminogen from these cells were also reduced relative to the wild-type. uPA could activate MAP kinases in the wild-type and two of the cell lines with the least uPA-binding, but the amount of the activated forms of the signalling molecules were reduced. Immunoblotting using two different anti-uPA receptor antibodies showed two cross-reacting protein species of approximately 53 kDa and approximately 38 kDa. The proportion of the lower Mr band to the higher Mr band was found to be reduced in all the cell lines relative to the wild-type. Chemical cross-linking with single-chain **urokinase** (scuPA) showed only one high-molecular-weight adduct, with Mr approximately 90 kDa, in all the cell lines tested. Similarly, cross-linking with the **amino terminal fragment** of uPA yielded a single approximately 70 kDa adduct. These would indicate that only the approximately 53 kDa band was responsible for cross-linking reactions. Equilibrium binding experiments showed that only one set of high-affinity binding sites for the wild-type cells. However, the binding of scuPA to two of these cell lines was best fitted to a two-site model, one of which was similar to the high-affinity binding sites of the wild-type, although the number of sites was reduced, while the other was of much lower affinity but was large in number. These results are discussed in relation to changes in the structure of ligand binding machinery in these cells, which affect other cellular functions.

L15 ANSWER 34 OF 129 MEDLINE on STN

2000424232. PubMed ID: 10943860. Cartilage degradation and invasion by rheumatoid synovial fibroblasts is inhibited by gene transfer of a cell surface-targeted plasmin inhibitor. van der Laan W H; Pap T; Ronday H K; Grimbergen J M; Huisman L G; TeKoppele J M; Breedveld F C; Gay R E; Gay S; Huizinga T W; Verheijen J H; Quax P H. (Gaubius Laboratory, The Netherlands Organization for Applied Scientific Research, Prevention and Health, and Leiden University Medical Center.) Arthritis and rheumatism, (2000 Aug) 43 (8) 1710-8. Journal code: 0370605. ISSN: 0004-3591. Pub. country: United States. Language: English.

AB OBJECTIVE: Joint destruction in rheumatoid arthritis (RA) is a result of degradation and invasion of the articular cartilage by the pannus tissue.

The present study was undertaken to examine the role of the plasminogen activation system in cartilage degradation and invasion by synovial fibroblasts and investigate a novel gene therapeutic approach using a cell surface-targeted plasmin inhibitor (**ATF**.BPTI). METHODS: Adenoviral vectors were used for gene transfer. The effects of **ATF**.BPTI gene transfer on RA synovial fibroblast-dependent cartilage degradation were studied in vitro, and cartilage invasion was studied in vivo in the SCID mouse coimplantation model. RESULTS: The results indicate that cartilage matrix degradation by rheumatoid synovial fibroblasts is plasmin mediated and depends on **urokinase**-type plasminogen activator for activation. Targeting plasmin inhibition to the cell surface of the fibroblasts by gene transfer of a cell surface-binding plasmin inhibitor resulted in a significant reduction of cartilage matrix degradation in vitro and of cartilage invasion in vivo. Compared with uninfected rheumatoid synovial fibroblasts, the mean +/-SEM cartilage degradation in vitro was reduced to 87.9+/-0.9% after LacZ gene transfer versus a reduction to 24.0+/-1.6% after **ATF**.BPTI gene transfer (P<0.0001). The mean +/- SEM in vivo cartilage invasion score was 3.1+/-0.4 in the control-transduced fibroblasts and 1.8+/-0.4 in the **ATF**.BPTI-transduced fibroblasts (P<0.05). CONCLUSION: These results indicate a role of the plasminogen activation system in synovial fibroblast-dependent cartilage degradation and invasion in RA, and demonstrate an effective way to inhibit this by gene transfer of a cell surface-targeted plasmin inhibitor.

L15 ANSWER 35 OF 129 MEDLINE on STN

2000179850. PubMed ID: 10713063. Recombinant toxins that bind to the **urokinase** receptor are cytotoxic without requiring binding to the alpha(2)-macroglobulin receptor. Rajagopal V; Kreitman R J. (Laboratory of Molecular Biology, Division of Basic Sciences, NCI, National Institutes of Health, Bethesda, Maryland 20892, USA.) Journal of biological chemistry, (2000 Mar 17) 275 (11) 7566-73. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The alpha(2-)macroglobulin receptor (alpha(2)MR) has been reported to mediate the internalization of the **urokinase** plasminogen activator receptor (uPAR) via ligand binding to both receptors. To target malignant uPAR-expressing cells and to determine whether uPAR can internalize without ligand binding to alpha(2)MR, we engineered two recombinant toxins, **ATF**-PE38 and **ATF**-PE38KDEL. Each consists of the **amino-terminal fragment** (**ATF**) of human **urokinase** and a truncated form of Pseudomonas exotoxin (PE) devoid of domain Ia, which binds alpha(2)MR. **ATF**-PE38 and **ATF**-PE38KDEL were cytotoxic toward malignant uPAR-bearing cells, with IC(50) values as low as 0.02 ng/ml (0.3 pM). Cytotoxicity could be blocked using either recombinant **urokinase** or free **ATF**, indicating that the cytotoxicity of the recombinant toxins was specific. Radiolabeled **ATF**-PE38 had high affinity for uPAR (K(d) = 0.4-8 nM) on a variety of different malignant cell types and internalized at a rate similar to that of **ATF**. The cytotoxicity was not diminished by receptor-associated protein, which binds and shields the alpha(2)MR from other proteins, or by incubation with phorbol myristate acetate, which is known to decrease the number of alpha(2)MRs in U937 cells or by antibodies to alpha(2)MR. Therefore, these recombinant toxins appear to internalize via uPAR without association with the alpha(2)MR.

L15 ANSWER 36 OF 129 MEDLINE on STN

2000125944. PubMed ID: 10657995. Cytosolic immunization allows the expression of preATF-saporin chimeric toxin in eukaryotic cells. Fabbrini M S; Carpani D; Soria M R; Ceriotti A. (Department of Biological and Technological Research-Dibit, San Raffaele Scientific Institute, 20132 Milano, Italy.. fabbrini.serena@hsr.it) . FASEB journal : official publication of the Federation of American Societies for Experimental Biology, (2000 Feb) 14 (2) 391-8. Journal code: 8804484. ISSN: 0892-6638. Pub. country: United States. Language: English.

AB In this work, we have devised an intracellular immunization strategy for the expression in high amounts of **ATF**-saporin, a targeted chimeric toxin constituted by the **ATF** receptor binding domain of human **urokinase** and the plant ribosome-inactivating protein saporin, which has been shown to

be highly cytotoxic to target cells. This strategy may allow the production of highly toxic secretory proteins in eukaryotic cells, avoiding cell suicide caused by autointoxication. The procedure consists of equipping host cells with cytosolic neutralizing antibodies directed toward the toxic domain of the heterologous polypeptide. We show that this intracellular immunization is essential for the synthesis of correctly folded, biologically active **ATF**-SAP in the high amounts needed to investigate its in vivo anti-metastatic potential. Such a strategy should be generally useful for the production of toxic molecules of therapeutic value whose folding and maturation require transit through the eukaryotic secretory pathway. Fabbrini, M. S., Carpani, D., Soria, M. R., Ceriotti, A. Cytosolic immunization allows the expression of preATF-saporin chimeric toxin in eukaryotic cells.

L15 ANSWER 37 OF 129 MEDLINE on STN

2000102888. PubMed ID: 10634825. Lysophosphatidylcholine induces **urokinase**-type plasminogen activator and its receptor in human macrophages partly through redox-sensitive pathway. Oka H; Kugiyama K; Doi H; Matsumura T; Shibata H; Miles L A; Sugiyama S; Yasue H. (Department of Cardiovascular Medicine, Kumamoto University School of Medicine, Kumamoto City, Japan.) Arteriosclerosis, thrombosis, and vascular biology, (2000 Jan) 20 (1) 244-50. Journal code: 9505803. ISSN: 1079-5642. Pub. country: United States. Language: English.

AB **Urokinase**-type plasminogen activator (uPA) and its cell surface receptor (uPAR) have been shown to be expressed in macrophages in atherosclerotic arterial walls, but the regulatory mechanisms of their expression remain unclear. The present study was performed to examine the effects of lysophosphatidylcholine (lysoPC), an important atherogenic lipid, on the expression of uPA and uPAR in human monocyte-derived macrophages. LysoPC upregulated the mRNA expression of uPA and uPAR, and it increased the protein expression of uPA in the culture medium and bound to the cell surface and of uPAR in the particulate fraction of the cells. LysoPC significantly increased the binding of the **amino-terminal fragment** of uPA to the treated cells and the cell-associated plasminogen activator activity. LysoPC stimulated superoxide anion production and increased intracellular oxidant levels in the cells. The combined incubation with reduced glutathione diethyl ester or N-acetylcysteine, antioxidants, suppressed the upregulation of uPA and uPAR mRNA and the increase in plasminogen activator activity by lysoPC. uPA and uPAR mRNA expression was also induced by the incubation with xanthine and xanthine oxidase, a superoxide anion-generating system. The results suggest that lysoPC increased the expression of uPA and uPAR and their functional activities in human monocyte-derived macrophages, at least in part through a redox-sensitive mechanism. This coordinate increase in the expression of uPA and uPAR in human macrophages by lysoPC could play an important role in plaque formation and disruption, arterial remodeling, and angiogenesis in atherosclerotic arterial walls.

L15 ANSWER 38 OF 129 MEDLINE on STN

2000075754. PubMed ID: 10609663. Systemic delivery of antiangiogenic adenovirus AdmATF induces liver resistance to metastasis and prolongs survival of mice. Li H; Griscelli F; Lindenmeyer F; Opolon P; Sun L Q; Connault E; Soria J; Soria C; Perricaudet M; Yeh P; Lu H. (CNRS UMR 1582/Rhone-Poulenc Rorer Gencell, Institut Gustave Roussy, Villejuif, France.) Human gene therapy, (1999 Dec 10) 10 (18) 3045-53. Journal code: 9008950. ISSN: 1043-0342. Pub. country: United States. Language: English.

AB Systemic administration of Ad5-based recombinant adenovirus leads to preferential transduction of the liver. Using this property, we have assessed the potential of venous viral injection to deliver a recombinant antiangiogenic adenovirus to treat cancer dissemination and improve survival. The results demonstrate that venous injection of adenovirus AdmATF, which encodes a secretable mouse **ATF** (**amino-terminal fragment** of **urokinase**) known to inhibit angiogenesis, suppressed angiogenesis induced by colon cancer metastasis growth in mice liver and improved survival. Nude mice were injected intravenously with 5 X 10(9)

pre of AdmATF and subsequently challenged after a 3 day interval by intrasplenically injected human colon carcinoma cells (LS174T, 3 x 10(6)) that home to liver. Microscopic inspection revealed that, within the AdmATF-pretreated mice (n = 8), the size and number of liver-metastasized nodules on day 30 were remarkably reduced (80% in number, $p < 0.05$) compared with control mice (n = 7) pretreated in parallel with a control adenovirus. Metastatic growth-related liver weight gain was also inhibited up to 90%. AdmATF-specific capability that offers liver resistance to the apparition and growth of liver metastasis was shown to correlate with the inhibition of peritumoral and intratumoral angiogenesis (reduced by 79%, $p < 0.01$ as shown by anti-vWF immunostaining of liver sections) and a twofold increase in tumor necrotic area and an eightfold increase in apoptotic tumor cell number. This protective effect was still observed when the mice were challenged 10 days after venous AdmATF injection (visible metastasis nodules: 6.3+/-3.1, n = 7 for control mice versus 2.7+/-2.9, n = 10 for treated mice, $p < 0.05$). More importantly, the mean survival has been prolonged from 45.1 days (n = 9) to 83.3 days (n = 10, $p < 0.05$). Altogether, the high efficacy, although transient, in this experimental mice model strongly advocates the plausibility of transforming the liver into a dissemination resistant organ by antiangiogenic gene therapy through systemic delivery approach.

L15 ANSWER 39 OF 129 MEDLINE on STN

2000033582. PubMed ID: 10564640. In vivo angiogenic activity of **urokinase**: role of endogenous fibroblast growth factor-2. Ribatti D; Leali D; Vacca A; Giuliani R; Gualandris A; Roncali L; Nolli M L; Presta M. (Institute of Human Anatomy, University of Bari, Piazza G. Cesare 11, Italy.) Journal of cell science, (1999 Dec) 112 (Pt 23) 4213-21. Journal code: 0052457. ISSN: 0021-9533. Pub. country: ENGLAND: United Kingdom. Language: English.

AB In vitro experimental evidences suggest that the proteolytic degradation of the extracellular matrix (ECM) by activation of the **urokinase**-type plasminogen activator (uPA)/plasmin system may affect growth factor activity and bioavailability. However, no direct in vivo observations were available to support this hypothesis. Here we demonstrate that endothelial GM 7373 cells overexpressing human uPA (uPA-R5 cells) cause the release of (125)I-labeled fibroblast growth factor-2 (FGF2) from endothelial ECM in a plasmin-dependent manner. Accordingly, uPA-R5 cells are angiogenic in vivo when applied on the top of the chorioallantoic membrane (CAM) of the chick embryo. In contrast, mock-transfected Neo2 cells are unable to release ECM-bound (125)I-FGF2 and are poorly angiogenic. Neovascularization elicited by uPA-R5 cells is significantly reduced by neutralizing anti-FGF2 antibodies to values similar to those observed in Neo2 cell-treated CAMs. Accordingly, purified human uPA stimulates neovascularization of the CAM in the absence of an inflammatory response. The angiogenic activity of uPA is significantly inhibited by neutralizing anti-FGF2 antibodies or by pretreatment with phenylmethylsulfonyl fluoride. The non-catalytic, receptor-binding **amino-terminal fragment** of uPA is instead non angiogenic. Taken together, the data indicate that uPA is able to induce angiogenesis in vivo via a plasmin-dependent degradation of ECM that causes the mobilization of stored endogenous FGF2.

L15 ANSWER 40 OF 129 MEDLINE on STN

2000005706. PubMed ID: 10537314. **Urokinase** receptor interacts with alpha(v)beta5 vitronectin receptor, promoting **urokinase**-dependent cell migration in breast cancer. Carriero M V; Del Vecchio S; Capozzoli M; Franco P; Fontana L; Zannetti A; Botti G; D'Aiuto G; Salvatore M; Stoppelli M P. (National Cancer Institute, Naples, Italy.. stoppelli@iigbna.iigb.na.cnr.it) . Cancer research, (1999 Oct 15) 59 (20) 5307-14. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB Perturbation of adhesive interactions at cell-substratum and cell-cell contact sites is a critical event in the multistep process of cancer invasion. Recent studies indicate that the **urokinase** receptor (uPAR) is associated in large molecular complexes with other molecules, such as

integrins. To test the possibility that uPAR may physically and functionally interact with vitronectin (Vn) receptors, we determined the expression level of uPAR, alpha(v)beta3, and alpha(v)beta5 Vn receptors in 10 human breast carcinomas. Here, we show the ability of uPAR to physically associate with alpha(v)beta5 in the breast carcinomas examined. The functional effects of this interaction were studied using HT1080 human fibrosarcoma and MCF-7 human breast carcinoma cell lines, both exhibiting a **urokinase**-dependent physical association between uPAR and alpha(v)beta5. Both cell lines respond to **urokinase** or to its noncatalytic **amino-terminal fragment** by exhibiting remarkable cytoskeletal rearrangements that are mediated by alpha(v)beta5 and require protein kinase C activity. On the contrary, binding of Vn to alpha(v)beta5 results in the protein kinase C-independent formation of F-actin containing microspike-type structures. Furthermore, alpha(v)beta5 is required for **urokinase**-directed, receptor-dependent MCF-7 and HT1080 cell migration. These data show that uPAR association with alpha(v)beta5 leads to a functional interaction of these receptors and suggest that uPAR directs cytoskeletal rearrangements and cell migration by altering alpha(v)beta5 signaling specificity.

L15 ANSWER 41 OF 129 MEDLINE on STN

1999384291. PubMed ID: 10454570. Role of distinct mitogen-activated protein kinase pathways and cooperation between Ets-2, **ATF**-2, and Jun family members in human **urokinase**-type plasminogen activator gene induction by interleukin-1 and tetradecanoyl phorbol acetate. Cirillo G; Casalino L; Vallone D; Caracciolo A; De Cesare D; Verde P. (International Institute of Genetics and Biophysics, CNR, 80125 Naples, Italy.) Molecular and cellular biology, (1999 Sep) 19 (9) 6240-52. Journal code: 8109087. ISSN: 0270-7306. Pub. country: United States. Language: English.

AB We have investigated the in vivo and in vitro regulation of the human **urokinase**-type plasminogen activator (uPA) gene by interleukin-1 (IL-1) and analyzed the transcription factors and signalling pathways involved in the response of the -2.0-kb uPA enhancer to IL-1 induction and to tetradecanoyl phorbol acetate (TPA) induction. Mutational analysis showed the cooperative activity of the Ets-binding site (EBS) and the two AP-1 elements of the enhancer. The results reveal that the EBS is required for the response to both inducers mediated by Ets-2, which is regulated at a level subsequent to DNA binding, by an IL-1- and phorbol ester-inducible transactivation domain. Both the IL-1 and the TPA-mediated induction result in a drastic increase of AP-1 binding to the downstream site of the enhancer (uPA 3' TPA-responsive element), while a mostly qualitative change, resulting from the interplay between **ATF**-2 homodimers and c-Jun-**ATF**-2 heterodimers, takes place at the upstream AP-1 element. The analysis of two distinct mitogen-activated protein kinase pathways shows that stress-activated protein kinase-Jun N-terminal kinase activation, resulting in the phosphorylation of **ATF**-2, c-Jun, and JunD, is required not only for the IL-1- but also for the TPA-dependent induction, while the extracellular signal-related kinase 1 (ERK-1) and ERK-2 activation is involved in the TPA- but not in the IL-1-dependent stimulation of the uPA enhancer.

L15 ANSWER 42 OF 129 MEDLINE on STN

1999318816. PubMed ID: 10388537. **Urokinase**-type plasminogen activator binding to its receptor stimulates tumor cell migration by enhancing integrin-mediated signal transduction. Yebra M; Goretzki L; Pfeifer M; Mueller B M. (Department of Immunology, The Scripps Research Institute, 10550 North Torrey Pines Road, IMM13, La Jolla, California, 92037, USA.) Experimental cell research, (1999 Jul 10) 250 (1) 231-40. Journal code: 0373226. ISSN: 0014-4827. Pub. country: United States. Language: English.

AB **Urokinase**-type plasminogen activator (uPA) and its receptor (uPAR) participate in matrix degradation and cell migration by focusing proteolysis and functioning as a signaling ligand/receptor complex. uPAR, anchored by a lipid moiety in the membrane, is thought to require a transmembrane adapter to transduce signals into the cytoplasm. To study uPAR signaling, we transfected the prostate carcinoma cell line LNCaP, which does not express endogenous uPA or uPAR, with a uPAR encoding cDNA,

resulting in high level surface expression. We studied migration of these cells on fibronectin, which is mediated by the integrin alpha5beta1. Ligation of uPAR with uPA or its **amino-terminal fragment** enhanced haptotactic migration to fibronectin. In cells on fibronectin, but not on poly-l-lysine, ligation of uPAR also resulted in tyrosine phosphorylation of several proteins, including two proteins involved in integrin signaling, focal adhesion kinase and the crk-associated substrate p130(Cas). Furthermore, after uPAR ligation, uPAR was co-immunoprecipitated with beta1 integrins from the detergent-insoluble fraction of cell lysates. Thus, our data suggest that uPAR occupancy results in an interaction between uPAR and integrins and a potentiation of integrin-mediated signaling, which leads to enhanced cell migration. Copyright 1999 Academic Press.

L15 ANSWER 43 OF 129 MEDLINE on STN

1999293019. PubMed ID: 10362798. Role and localization of **urokinase** receptor in the formation of new microvascular structures in fibrin matrices. Kroon M E; Koolwijk P; van Goor H; Weidle U H; Collen A; van der Pluijm G; van Hinsbergh V W. (Gaubius Laboratory, Leiden University Hospital, Groningen Leiden The Netherlands.) American journal of pathology, (1999 Jun) 154 (6) 1731-42. Journal code: 0370502. ISSN: 0002-9440. Pub. country: United States. Language: English.

AB Fibrin or a fibrinous exudate can facilitate angiogenesis in many pathological conditions. In vitro, the outgrowth of capillary-like structures in fibrin can be mimicked by exposing human microvascular endothelial cells (hMVECs) to an angiogenic growth factor and tumor necrosis factor (TNF)-alpha. **Urokinase**-type plasminogen activator (u-PA) and plasmin activities are required for this angiogenic process. This study focuses on the role and localization of the u-PA receptor (u-PAR) in newly formed microvascular structures. The u-PAR-blocking monoclonal antibody (MAb) H-2 completely inhibited the formation of capillary-like tubular structures induced by exposure of hMVECs to basic fibroblast growth factor and TNF-alpha. This was accompanied by a several-fold increase in u-PA accumulation in the conditioned medium. The effect of MAb H-2 was not caused by blocking cellular activation by u-PA/u-PAR interaction, as the **amino-terminal fragment** (**ATF**) of u-PA, which also activates u-PAR, prevented tube formation. In addition, the inhibition by MAb H-2 was not due to an effect of the antibody on u-PAR-vitronectin binding. These data show that inhibition of tube formation can be caused not only by inhibition of u-PA or plasmin activities but also by unavailability of the u-PAR for cell-bound proteolysis. Immunohistochemical analysis showed that in in vitro angiogenesis u-PAR and u-PA were localized on the invading, tube-forming hMVECs and not on the endothelial cells that are located on top of the fibrin matrix. u-PAR and u-PA were also prominently expressed on endothelial cells of neovessels present in an atherosclerotic plaque. These data may give more insight into the role of u-PAR in repair-associated angiogenesis.

L15 ANSWER 44 OF 129 MEDLINE on STN

1999272427. PubMed ID: 10339491. Characterization of cell-associated plasminogen activation catalyzed by **urokinase**-type plasminogen activator, but independent of **urokinase** receptor (uPAR, CD87). Longstaff C; Merton R E; Fabregas P; Felez J. (The National Institute for Biological Standards and Control, South Mimms, Hertfordshire, UK.. clongstaff@nibsc.ac.uk) . Blood, (1999 Jun 1) 93 (11) 3839-46. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB The 55-kD **urokinase** (uPA) receptor (uPAR, CD87) is capable of binding uPA and may be involved in regulating cell-associated plasminogen activation and pericellular proteolysis. While investigating the relationship between uPAR levels and plasmin generation, we found that uPA-catalyzed plasminogen activation is stimulated by cells which do not express uPAR. This uPAR-independent mechanism appears to be at least as effective in vitro as uPAR-dependent stimulation, such that stimulation on the order of 30-fold was observed, resulting from improvements in both

apparent Kcat and apparent Km. The mechanism depends on simultaneous binding of both uPA and plasminogen to the cell and requires the presence of the **amino-terminal fragment** (**ATF**), available in single chain and two chain high-molecular-weight uPA, but not low-molecular-weight uPA. Stimulation was observed in all leukemic cell lines investigated at similar optimum concentrations of 10(6) to 10(7) cells/mL and may be more general. A mechanism is proposed whereby uPA can associate with binding sites on the cell surface of lower affinity, but higher capacity than uPAR, but these are sufficient to stimulate plasmin generation even at subphysiologic uPA concentrations. This mechanism is likely to operate under conditions commonly used for in vitro studies and may have some significance in vivo.

L15 ANSWER 45 OF 129 MEDLINE on STN

1999257884. PubMed ID: 10326034. Adenovirus-mediated delivery of a uPA/uPAR antagonist suppresses angiogenesis-dependent tumor growth and dissemination in mice. Li H; Lu H; Griscelli F; Opolon P; Sun L Q; Ragot T; Legrand Y; Belin D; Soria J; Soria C; Perricaudet M; Yeh P. (CNRS-Rhone-Poulenc Rorer-IGR UMR 1582, Institut Gustave Roussy, Villejuif, France.) Gene therapy, (1998 Aug) 5 (8) 1105-13. Journal code: 9421525. ISSN: 0969-7128. Pub. country: ENGLAND: United Kingdom. Language: English.

AB AdmATF is a recombinant adenovirus encoding a secreted version of the **amino-terminal fragment** (**ATF**) of murine **urokinase** (uPA). This defective adenovirus was used in three murine models to assess the antitumoral effects associated with local or systemic delivery of **ATF**, a broad cell invasion inhibitor that antagonizes uPA binding to its cell surface receptor (uPAR). A single intratumoral injection of AdmATF into pre-established MDA-MB-231 human breast xenografts grown in athymic mice, or into pre-established C57/BL6 syngeneic Lewis lung carcinoma resulted in a specific arrest of tumor growth. Neovascularization within and at the vicinity of the injection site was also suppressed, suggesting that AdmATF inhibited primary tumor growth by targeting angiogenesis. AdmATF also interfered with tumor cell establishment at distant sites: (1) lung dissemination of Lewis lung carcinoma cells was significantly reduced following intratumoral injection at the primary site; and (2) systemic administration of AdmATF inhibited subsequent liver metastasis in a LS174T human colon carcinoma xenograft model. These data outline the potential of using a recombinant adenovirus directing the secretion of an antagonist of cell-associated uPA for cancer gene therapy.

L15 ANSWER 46 OF 129 MEDLINE on STN

1999163976. PubMed ID: 10066093. **Urokinase** induces receptor mediated brain tumor cell migration and invasion. MacDonald T J; DeClerck Y A; Laug W E. (Department of Pediatrics, Childrens Hospital Los Angeles University of Southern California School of Medicine, 90027, USA.) Journal of neuro-oncology, (1998 Dec) 40 (3) 215-26. Journal code: 8309335. ISSN: 0167-594X. Pub. country: Netherlands. Language: English.

AB The plasminogen activation (PA) system plays an important role in tumor invasion by initiating pericellular proteolysis of the extracellular matrix (ECM) and inducing cell migration. Malignant brain tumors overexpress PA members and characteristically invade by migrating on ECM-producing white matter tracts and blood vessel walls. To determine whether **urokinase**-type plasminogen activator (uPA) and its receptor (uPAR) directly modulate the migration of brain tumor cells, we examined six human brain tumor cell lines, 2 astrocytomas (SW1088, SW1783), 2 medullobastomas (Daoy, D341Med), and 2 glioblastomas (U87MG, U118MG), for their surface uPAR expression, endogenous PA activity, and functional proteolytic activity by an ECM-degradation assay. Migration on Transwell membranes and invasion of Matrigel was then tested by pre-incubating the cells with increasing concentrations of either uPA, the proteolytically inactive **amino-terminal fragment** (**ATF**) of uPA, or the uPAR cleaving enzyme, phosphatidylinositol-specific phospholipase C (PI-PLC). All of the cell lines, except D341Med, express surface uPAR protein and uPA activity. High levels of uPAR and uPA activity correlated with cellular degradation of ECM, cell migration, and Matrigel invasion. Cell

migration and invasion were enhanced by uPA or **ATF** in a dose dependent manner, while PI-PLC treatment abolished the uPA effect and inhibited migration and invasion. We conclude that ligation of uPAR by uPA directly induces brain tumor cell migration, independent of uPA-mediated proteolysis; and in concert with ECM degradation, markedly enhances invasion. Conversely, removing membrane bound uPAR from the surface of the cells studied inhibited their ability to migrate and invade even in the presence of proteolytically active uPA.

L15 ANSWER 47 OF 129 MEDLINE on STN

1999141290. PubMed ID: 9974409. **Urokinase** activates the Jak/Stat signal transduction pathway in human vascular endothelial cells. Dumler I; Kopmann A; Weis A; Mayboroda O A; Wagner K; Gulba D C; Haller H. (Franz Volhard Clinic and Max-Delbruck Center for Molecular Medicine, Virchow Klinikum-Charite, Humboldt University of Berlin,Germany.. dumler@fvk-berlin.de) . Arteriosclerosis, thrombosis, and vascular biology, (1999 Feb) 19 (2) 290-7. Journal code: 9505803. ISSN: 1079-5642. Pub. country: United States. Language: English.

AB Endothelial cells demonstrate high **urokinase** expression and upregulation of **urokinase** receptors in response to vascular injury. **Urokinase** receptor binding facilitates endothelial cell migration into an arterial wound; however, the signaling cascade induced by the **urokinase** receptor in this cell type is incompletely understood. Because the Janus kinase (Jak)/signal transducer and activator of transcription (Stat) pathway seems to be important for vessel function, we investigated the hypothesis that **urokinase** receptor binding activates Jak/Stat signaling in human vascular endothelial cells. Incubation of endothelial cells with **urokinase**-type plasminogen activator (uPA,1 nmol/L) induced a rapid and pronounced increase in tyrosine phosphorylation of several proteins with a molecular weight between 80 to 90 and 130 to 140 kDa. The same pattern of tyrosine phosphorylation was found after treatment with 1 nmol/L **ATF**, the **urokinase amino-terminal fragment**, which is devoid of proteolytic activity but still binds to the **urokinase** receptor. Using coimmunoprecipitation techniques, we demonstrated that the activated **urokinase** receptor is associated with 2 cytoplasmic tyrosine kinases of the Jak family, viz, Jak1 and Tyk2. uPA and **ATF** induced a time-dependent activation of both kinases, as shown by immunoprecipitation and Western blot analysis. Using electrophoretic mobility shift and supershift assays, we then demonstrated that Stat1 is rapidly activated in endothelial cells in response to uPA and **ATF**. Furthermore, Stat1 specifically binds to the regulatory elements interferon-gamma activation site/interferon-stimulated response element. The uPA-induced, time-dependent translocation of Stat1 to cell nuclei was confirmed by confocal microscopy study and immunoblotting of nuclear extracts with an anti-Stat1 antibody. This study provides evidence for a novel signaling pathway for uPA in human vascular endothelial cells. Direct activation of the Jak/Stat system via the uPA-receptor complex may be an important mechanism for endothelial cell migration and/or proliferation during angiogenesis and after vascular injury.

L15 ANSWER 48 OF 129 MEDLINE on STN

1999111061. PubMed ID: 9815812. Vitronectin binding to **urokinase** receptor in human breast cancer. Carriero M V; Del Vecchio S; Franco P; Potena M I; Chiaradonna F; Botti G; Stoppelli M P; Salvatore M. (Istituto Nazionale per lo Studio e la Cura dei Tumori, Via M. Semmola.) Clinical cancer research : an official journal of the American Association for Cancer Research, (1997 Aug) 3 (8) 1299-308. Journal code: 9502500. ISSN: 1078-0432. Pub. country: United States. Language: English.

AB Functional assembly of the plasminogen-dependent proteolytic system on the cell surface requires multiple interactions involving **urokinase** (uPA), **urokinase** receptor (uPAR), plasminogen activator inhibitors, and other molecules that mediate cell migration and adhesion. We analyzed the in vitro interaction of uPAR-containing particulate cell fractions with the **amino-terminal fragment** (**ATF**) of human **urokinase** and the matrix-like form of vitronectin. Binding and cross-linking of 125I-labeled **ATF** to crude membrane extracts from LB6-19 mouse cells

overexpressing human uPARs in the presence of 2.5 mM urea denatured vitronectin led to the formation of Mr 137,000, 92, 000, and 82,000 covalent complexes. Immunoprecipitation of the preformed cross-linked 125I-labeled complexes with anti-vitronectin, anti-uPA, or anti-uPAR antibodies revealed that the Mr 82,000 and 92, 000 species do contain **ATF** and vitronectin and identified the Mr 137, 000 species as a ternary complex formed by **ATF**, uPAR, and vitronectin. A similar electrophoretic pattern was displayed by acid-pretreated membranes extracted from MCF-7 breast carcinoma or HT1080 fibrosarcoma cell lines, as well as a ductal breast carcinoma specimen; the latter exhibited complex formation at concentrations of vitronectin lower than 10 nM. Finally, uPAR-vitronectin interaction was further documented by the decreased reactivity of an anti-uPAR polyclonal antibody to acid-pretreated sections of 10 breast carcinomas that had been preincubated with vitronectin. Our findings highlight the ability of uPAR to interact simultaneously with vitronectin and uPA in breast cancer, supporting a dynamic coupling of the molecular mechanisms underlying plasminogen-dependent matrix degradation and cell adhesion.

L15 ANSWER 49 OF 129 MEDLINE on STN

1999101360. PubMed ID: 9886269. Suppression of keratinocyte proliferation by plasminogen activator inhibitor-2. Hibino T; Matsuda Y; Takahashi T; Goetinck P F. (Cutaneous Biology Research Center, Department of Dermatology, Massachusetts General Hospital and Harvard Medical School, Charlestown, USA.) Journal of investigative dermatology, (1999 Jan) 112 (1) 85-90. Journal code: 0426720. ISSN: 0022-202X. Pub. country: United States. Language: English.

AB We have previously shown that **urokinase** plasminogen activator (uPA) stimulates the growth of human keratinocytes in culture. For this effect, uPA activity is essential to generate the active **amino terminal fragment**, by an autolytic process. Our findings indicated further that inhibition of uPA may result in the suppression of growth of keratinoytes. Here, we provide evidence that plasminogen activator inhibitor (PAI)-2 has an anti-proliferative effect on keratinocytes. The uPA activity in cultured keratinocytes increased in parallel with cell proliferation, reaching a maximum level at confluency and decreasing gradually thereafter. The analysis of synchronized cells showed that the peak uPA activity in the medium occured just prior to S-phase, suggesting that the production and secretion of uPA is related to cell proliferation. In contrast, PAI-2 levels showed a steady increase, even after confluency. When PAI-2, purified from human cornified cells, was added to synchronized keratinocytes, S-phase was no longer evident and the peak uPA activity was eliminated. In experiments with a bacterially expressed PAI-2 fusion protein, [3H]thymidine incorporation by keratinocytes was significantly suppressed, confirming an anti-proliferative effect of PAI-2. These results strongly suggest that PAI-2 is involved in the regulation of keratinocyte proliferation and differentiation.

L15 ANSWER 50 OF 129 MEDLINE on STN

1999065689. PubMed ID: 9848876. **Urokinase** receptor-dependent upregulation of smooth muscle cell adhesion to vitronectin by **urokinase**. Chang A W; Kuo A; Barnathan E S; Okada S S. (University of Pennsylvania School of Medicine, Philadelphia, USA.) Arteriosclerosis, thrombosis, and vascular biology, (1998 Dec) 18 (12) 1855-60. Journal code: 9505803. ISSN: 1079-5642. Pub. country: United States. Language: English.

AB The plasminogen activator system has been implicated in the modulation of the response to vascular injury. Although **urokinase**-type plasminogen activator (uPA) and its receptor (uPAR) may enhance matrix degradation as well as migration and invasion by smooth muscle cells (SMCs), their roles in cell adhesion are uncertain. Therefore, we examined the ability of uPA and uPAR to modulate adhesion of cultured human vascular SMCs to various matrices. We demonstrated a dose-dependent stimulation of adhesion by single-chain uPA (scuPA) to vitronectin (maximum 1.55-fold [+/-0. 04-fold] increase, 10 nmol/L, $P<0.002$) but not to laminin, collagen I, or collagen IV. Baseline adhesion to vitronectin was completely inhibited by both EDTA and RGD peptide but was restored to >40% of control in the presence

of scuPA (P=0.001 and 0.040, respectively). Adhesion to vitronectin was also significantly enhanced by the **amino-terminal fragment** of uPA (P=0.007) and two-chain, high-molecular-weight uPA (P<0.01) but not by the low-molecular-weight fragment of uPA, which lacks the receptor-binding domain. Aprotinin, a plasmin inhibitor, had no effect on baseline or scuPA-stimulated adhesion, suggesting a plasmin-independent process. Preincubation of scuPA with soluble uPAR inhibited scuPA stimulation of adhesion by 88+/-14% (P=0.01), as did pretreatment of SMCs with phosphatidylinositol-specific phospholipase C, which removes glycophosphatidylinositol-anchored proteins, including uPAR. Antibodies to both alphavbeta3 and alphavbeta5 integrin inhibited baseline adhesion but not scuPA stimulation. Finally, coating plates with scuPA alone enabled cell adhesion, which could be inhibited by both soluble uPAR and anti-uPAR antibodies. These data suggest that uPA stimulates adhesion of SMCs specifically to vitronectin and that it is mediated by an interaction with uPAR. Upregulation of both proteins after vascular injury may facilitate migration through stimulation of both matrix degradation and cell adhesion.

L15 ANSWER 51 OF 129 MEDLINE on STN

1999057882. PubMed ID: 9837898. Mitogenic effects of **urokinase** on melanoma cells are independent of high affinity binding to the **urokinase** receptor. Koopman J L; Slomp J; de Bart A C; Quax P H; Verheijen J H. (Gaubius Laboratory, TNO Prevention and Health, 2301 CE Leiden, The Netherlands.) Journal of biological chemistry, (1998 Dec 11) 273 (50) 33267-72. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The structural and functional properties of the **urokinase**-type plasminogen activator (u-PA) that are involved in the mitogenic effect of this proteolytic enzyme on human melanoma cells M14 and IF6 and the role of the u-PA receptor (u-PAR) in transducing this signal were analyzed. Native u-PA purified from urine induced a mitogenic response in quiescent IF6 and M14 cells that ranged from 25 to 40% of the mitogenic response obtained by fetal calf serum. The half-maximum response in M14 and IF6 cells was reached at u-PA concentrations of approximately 35 and 60 nM, respectively. Blocking the proteolytic activity of u-PA resulted in a 30% decrease of the mitogenic effect, whereas inhibition of plasmin activity did not alter the mitogenic effect. No mitogenic response was elicited by low molecular weight u-PA, lacking the growth factor domain and the kringle domain. The **ATF** domain of u-PA induced a mitogenic response that was similar to complete u-PA. Defucosylated **ATF** and recombinant u-PA purified from Escherichia coli lacking all post-translational modifications did not induce a mitogenic response. Blocking the interaction of u-PA with u-PAR, using a specific monoclonal antibody, did not alter the mitogenic effect induced by u-PA. The binding of radiolabeled u-PA to M14 and IF6 cells was characterized by high affinity binding mediated by u-PAR and low affinity binding to an unknown binding site. These results demonstrate that proteolytically inactive u-PA is able to induce a mitogenic response in quiescent melanoma cells in vitro by a mechanism that involves the **ATF** domain but is independent of high affinity binding to u-PAR. Furthermore, it suggests that u-PA is able to bind with low affinity to a hitherto unidentified membrane associated protein that could be involved in u-PA-induced signal transduction.

L15 ANSWER 52 OF 129 MEDLINE on STN

1999037729. PubMed ID: 9821967. **Urokinase** induces proliferation of human ovarian cancer cells: characterization of structural elements required for growth factor function. Fischer K; Lutz V; Wilhelm O; Schmitt M; Graeff H; Heiss P; Nishiguchi T; Harbeck N; Kessler H; Luther T; Magdolen V; Reuning U. (Frauenklinik der Technischen Universitat Munchen, Klinikum rechts der Isar, Munich, Germany.) FEBS letters, (1998 Oct 30) 438 (1-2) 101-5. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB Ovarian cancer metastasis is associated with an increase in the **urokinase**-type plasminogen activator (uPA) and its receptor uPAR. We present evidence that binding of uPA to uPAR provokes a mitogenic response

in the human ovarian cancer cell line OV-MZ-6 in which endogenous uPA production had been significantly reduced by stable uPA 'antisense' transfection. High molecular weight (HMW) uPA, independent of its enzymatic activity, produced an up to 95% increase in cell number concomitant with 2-fold elevated [3H]thymidine incorporation as did the catalytically inactive but uPAR binding **amino-terminal fragment** of uPA, **ATF**. uPA-induced cell proliferation was significantly decreased by blocking uPA/uPAR interaction by the monoclonal antibody IIIF10 and by soluble uPAR. The efficiency of the uPAR binding synthetic peptide cyclo19,31 uPA19-31 to enhance OV-MZ-6 cell growth proved this molecular domain to be the minimal structural determinant for uPA mitogenic activity. Dependence of uPA-provoked cell proliferation on uPAR was further demonstrated in Raji cells which do not express uPAR and were thus not induced by uPA. However, upon transfection with full-length uPAR, Raji cells acquired a significant growth response to HMW uPA and **ATF**.

L15 ANSWER 53 OF 129 MEDLINE on STN

1998430982. PubMed ID: 9760182. Analysis of the ternary complex formation of human **urokinase** with the separated two domains of its receptor. Oda M; Shiraishi A; Hasegawa M. (Tokyo Research Laboratories, Kyowa Hakko Kogyo Co., Ltd, Japan.. masayuki.oda@kyowa.co.jp) . European journal of biochemistry / FEBS, (1998 Sep 1) 256 (2) 411-8. Journal code: 0107600. ISSN: 0014-2956. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Human **urokinase**-type-plasminogen-activator receptor (uPAR) is a glycolipid-anchored membrane glycoprotein comprising three structurally similar domains. We have succeeded in direct observation of the ternary complex formation of single-chain **urokinase** (scuPA) or its N-terminal fragment (**ATF**) with the separated domain-1 (N-terminal domain) and domain-(2+3) (internal and C-terminal domain) of human uPAR, by means of gel-filtration HPLC analysis. This complex was found to consist of the three components in an equimolar ratio (thus referred to as the three-part complex). To determine the nature of the interaction between these components, cross-linking experiments involving various kinds of cross-linkers and competitive binding assay on ELISA were performed. These experiments have shown that each uPAR domain can bind directly to scuPA at low affinity, and that both these domains contribute to the high-affinity binding between scuPA and uPAR in a synergistic manner. It can be considered that the synergistic effect of domain-1 and domain-(2+3) on scuPA binding would result from a conformational change, and that this steric event might trigger the signal transduction reported for scuPA/uPAR binding.

L15 ANSWER 54 OF 129 MEDLINE on STN

1998316656. PubMed ID: 9654084. A bifunctional hybrid molecule of the **amino-terminal fragment** of **urokinase** and domain II of bikunin efficiently inhibits tumor cell invasion and metastasis. Kobayashi H; Sugino D; She M Y; Ohi H; Hirashima Y; Shinohara H; Fujie M; Shibata K; Terao T. (Department of Obstetrics and Gynecology, Hamamatsu University, Shizuoka, Japan.) European journal of biochemistry / FEBS, (1998 May 1) 253 (3) 817-26. Journal code: 0107600. ISSN: 0014-2956. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Urinary trypsin inhibitor (UTI) inhibits efficiently tumor cell invasion and the formation of metastasis. The anti-metastatic effect is dependent on the COOH-terminal domain II of UTI [UTI-(78-136)-peptide]. To develop a molecule that binds with high affinity to the **urokinase** (uPA) receptor (uPAR) on tumor cell surfaces, a bifunctional hybrid molecule [uPA-(1-134)-UTI-(78-136)] consisting of the uPAR-binding NH2-terminal fragment [UTI-(78-136)-peptide] of uPA at the NH2-terminus of UTI-(78-136)-peptide was produced in Escherichia coli by genetic engineering. The purified hybrid protein inhibited trypsin and plasmin 2-3-fold less effectively than UTI-(78-136)-peptide and was found to bind to human tumor cells via uPAR, which was confirmed by cell binding and competition experiments. Using a modified Boyden chamber and an artificial basement membrane, Matrigel, it was found that the hybrid protein is very effective at inhibiting invasion by uPAR-expressing human

tumor cells. Sensitivities of tumor cells towards the anti-invasive effect of uPA-(1-134)-UTI-(78-136) correlated with the density of uPAR on human tumor cells. Furthermore, in the spontaneous metastasis model, the hybrid protein inhibited the formation of lung and/or lymphatic metastasis by human ovarian carcinoma and choriocarcinoma cells. The hybrid protein was much more effective than uPA-(1-134)-peptide, UTI-(78-136)-peptide, or UTI. We conclude that this approach extends the possibility of applying recombinant protein for therapeutic use in inhibition of human tumor cell metastasis.

L15 ANSWER 55 OF 129 MEDLINE on STN
1998231068. PubMed ID: 9569610. Production of a hybrid protein consisting of the N-terminal fragment of **urokinase** and the C-terminal domain of urinary trypsin inhibitor in Escherichia coli. Sugino D; Okushima M; Kobayashi H; Terao T. (Nissin Central Research Institute, Shiga, Japan.) Biotechnology and applied biochemistry, (1998 Apr) 27 (Pt 2) 145-52. Journal code: 8609465. ISSN: 0885-4513. Pub. country: ENGLAND: United Kingdom. Language: English.
AB We have constructed a hybrid protein (ATFHI) consisting of an N-terminal fragment from **urokinase** (**ATF**) and HI-8, which is the C-terminal domain of urinary trypsin inhibitor. The fusion genes for the hybrid proteins were engineered by PCR and cloned into expression plasmids. Under the control of the tac promoter, fusion genes were efficiently expressed in Escherichia coli. The hybrid proteins, produced as inclusion bodies in E. coli, were refolded by a dialysis method and purified by ion-exchange chromatography. ATFHI exhibited bifunctional activity related to antimetastatic effects: the **urokinase** receptor-binding activity of **ATF** and the inhibitory activity of HI-8 on plasmin.

L15 ANSWER 56 OF 129 MEDLINE on STN
1998192651. PubMed ID: 9525964. Binding of **urokinase**-type plasminogen activator to its receptor in MCF-7 cells activates extracellular signal-regulated kinase 1 and 2 which is required for increased cellular motility. Nguyen D H; Hussaini I M; Gonias S L. (Department of Biochemistry, University of Virginia Health Sciences Center, Charlottesville, Virginia 22908, USA.) Journal of biological chemistry, (1998 Apr 3) 273 (14) 8502-7. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.
AB Binding of **urokinase**-type plasminogen activator (uPA) to its receptor, uPAR, regulates cellular adhesion, migration, and tumor cell invasion. Some of these activities may reflect the ability of uPAR to initiate signal transduction even though this receptor is linked to the plasma membrane only by a glycosylphosphatidylinositol anchor. In this study, we demonstrated that single-chain uPA activates extracellular signal-regulated kinase 1 (ERK1) and ERK2 in MCF-7 breast cancer cells. Phosphorylation of ERK1 and ERK2 was increased 1 min after adding uPA and returned to baseline levels by 5 min. The **amino-terminal fragment** (**ATF**) of uPA, which binds to uPAR but lacks proteinase activity, also activated ERK1 and ERK2. Responses to uPA and **ATF** were eliminated when the cells were pretreated with PD098059, an inhibitor of mitogen-activated protein kinase kinase. uPA and **ATF** promoted the migration of MCF-7 cells across serum-coated Transwell membranes in vitro. Migration was increased 2.1 +/- 0.4-fold when uPA was added to the top chamber, 4. 8 +/- 0.8-fold when uPA was added to the bottom chamber, and 7.7 +/- 1.0-fold when uPA was added to both chambers. MCF-7 cells that were pulse-exposed to uPA for 30 min, and then washed to remove unbound ligand, demonstrated increased motility even though migration was allowed to occur for 24 h. PD098059 completely neutralized the effects of uPA on MCF-7 cellular motility, irrespective of whether the uPA was present for the entire motility assay or administered by pulse-exposure. These results demonstrate a novel, receptor-dependent signaling activity which is required for uPA-stimulated breast cancer cell migration.

L15 ANSWER 57 OF 129 MEDLINE on STN
1998073730. PubMed ID: 9409265. Induction of vascular SMC proliferation by **urokinase** indicates a novel mechanism of action in vasoproliferative

disorders. Kanse S M, Benzakour O, Kanthou C, Rost C, Bijnen H K, Preissner K T. (Max-Planck-Institute, Kerckhoff-Klinik, Bad Nauheim, Germany.. sandip.kanse@kerckhoff.med.uni-giessen.de) . Arteriosclerosis, thrombosis, and vascular biology, (1997 Nov) 17 (11) 2848-54. Journal code: 9505803. ISSN: 1079-5642. Pub. country: United States. Language: English.

AB The **urokinase**-type plasminogen activator (UPA) and its receptor are expressed in the vasculature and are involved in cell migration and remodeling of the extracellular matrix in the neointima. Vessels with atherosclerosis or neointimal hyperplasia, when compared with normal vessels, contain high UPA activity as well as increased levels of UPA receptor. In this study, we have identified the stimulation of vascular smooth muscle cell proliferation as a novel activity for UPA in the vessel wall. High-molecular-weight-UPA (12-200 nmol/L range) stimulated DNA synthesis and cell proliferation, which was half that induced by fetal calf serum or by platelet-derived growth factor-BB. UPA did not induce growth of endothelial cells, and tissue-type plasminogen activator showed no activity on either cell type. Induction of proliferation required the complete UPA molecule but was independent of the proteolytic activity of UPA, whereas neither the **amino-terminal fragment** nor the catalytic domain by itself was mitogenic. UPA also stimulated c-fos/c-myc mRNA expression and mitogen-activated protein kinase activity in smooth muscle cells. Blocking monoclonal antibodies against the UPA receptor and the enzymatic removal of receptors were ineffective in inhibiting the mitogenic effect of UPA, suggesting a UPA receptor-independent mechanism. Thus, we provide evidence for a novel function of UPA on vascular smooth muscle cell proliferation that, together with its previously documented involvement in regulating pericellular proteolysis-related events and cell migration, provides additional evidence for a role in the pathogenesis of atherosclerosis/restenosis.

L15 ANSWER 58 OF 129 MEDLINE on STN

1998072443. PubMed ID: 9409785. Cooperation of two PEA3/AP1 sites in uPA gene induction by TPA and FGF-2. D'Orazio D; Besser D; Marksitzer R; Kunz C; Hume D A; Kiefer B; Nagamine Y. (Friedrich Mieschner Institute, Basel, Switzerland.) Gene, (1997 Nov 12) 201 (1-2) 179-87. Journal code: 7706761. ISSN: 0378-1119. Pub. country: Netherlands. Language: English.

AB We have previously shown in NIH 3T3 fibroblasts that treatment with 12-O-tetradecanoylphorbol 13-acetate (TPA) or fibroblast growth factor-2 (FGF-2) activates the Ras/Erk signaling pathway in NIH 3T3 fibroblasts, leading to the induction of the **urokinase**-type plasminogen activator (uPA) gene. In this study, we characterize cis-acting elements involved in this induction. DNase I hypersensitive (HS) site analysis of the uPA promoter showed that two regions were enhanced after TPA and FGF-2 treatment. One was located 2.4kb upstream of the transcription start site (-2.4kb), where a known PEA3/AP1 (AGGAAATGAGGTCAT) element is located. The other was located in a previously undefined far upstream region. Sequencing of this region revealed a similar AP1/PEA3 (GTGATTCACTTCCT) element at -6.9 kb corresponding to the HS site. Deletion analysis of the uPA promoter in transient transfection assays showed that both PEA3/AP1 elements are required for full inducibility, suggesting a synergism between the two elements. When the two sites were inserted together upstream of a minimal promoter derived from the thymidine kinase gene, expression of the reporter gene was more strongly induced by TPA and FGF-2 than with either of the two elements alone. Alone, the -6.9 element was more potent than the -2.4 element. The involvement of AP1 as well as Ets transcription factors was confirmed by examining different promoter constructs containing deletions in either the AP-1 or the PEA3 element, and by using an expression plasmid for dominant negative Ets-2. Electromobility shift analyses using specific antibodies showed that c-Jun and, JunD bind to both elements with or without induction. In addition, **ATF**-2 binds to the -2.4-kb element even without induction and c-Fos to the -6.9-kb element only after induction. Accordingly, overexpression of c-Fos caused induction from the -6.9-kb element, but reduced induction from the -2.4-kb element. The involvement of the Ets-2 transcription factor was shown by using expression plasmids for wild-type and dominant

L15 ANSWER 59 OF 129 MEDLINE on STN

1998033175. PubMed ID: 9367352. The **amino-terminal fragment** of human **urokinase** directs a recombinant chimeric toxin to target cells: internalization is toxin mediated. Fabbrini M S; Carpani D; Bello-Rivero I; Soria M R. (Dibit-Department of Biological and Technological Research, San Raffaele Scientific Institute, Milano, Italy.. fabbris@dibit.hsr.it) . FASEB journal : official publication of the Federation of American Societies for Experimental Biology, (1997 Nov) 11 (13) 1169-76. Journal code: 8804484. ISSN: 0892-6638. Pub. country: United States. Language: English.

AB In contrast to two-chain **urokinase** (uPA), a chemical conjugate between uPA and native saporin (a cytotoxic plant seed ribosome-inactivating protein) did not require plasminogen activator inhibitors to be internalized. To dissect this pathway, we constructed a chimera consisting of the **amino-terminal fragment** (**ATF**) of human **urokinase** fused to a saporin isoform (SAP-3). The chimeric **ATF**-SAP toxin was expressed in Escherichia coli, purified, and characterized for its ribosome-inactivating activity. Besides being a potent inhibitor of protein synthesis in cell-free assays, **ATF**-SAP was specifically cytotoxic toward cells expressing human uPAR. Competition experiments indicated that both the human uPAR and the LDL-related receptor protein are involved in mediating the cell killing ability of **ATF**-SAP. We conclude that neither plasminogen activator inhibitors nor the catalytic moiety of **urokinase** are necessary to initiate these internalization pathways. Thus, saporin may play a role similar to plasminogen activator inhibitors in its ability to trigger internalization of uPAR-bound ligands through endocytic receptors.

L15 ANSWER 60 OF 129 MEDLINE on STN

1998026748. PubMed ID: 9362425. Mechanisms of the development of osteoblastic metastases. Goltzman D. (Department of Medicine, Royal Victoria Hospital and McGill University, Montreal, Quebec, Canada.) Cancer, (1997 Oct 15) 80 (8 Suppl) 1581-7. Ref: 29. Journal code: 0374236. ISSN: 0008-543X. Pub. country: United States. Language: English.

AB Although several neoplasms may produce osteoblastic metastases, carcinoma of the prostate is by far the most common. Biochemical and histologic studies indicate that osteolysis also is a manifestation of prostate carcinoma. Furthermore, factors such as parathyroid hormone-related peptide, which mediate osteolysis in other cancers, also appear to be operative in the bone breakdown induced by prostate carcinoma. However, the most unique skeletal effect of this tumor is its consistent capacity to stimulate osteoblasts to deposit new bone. Several bone growth factors have been detected in prostatic tissue and may contribute to this process. These include transforming growth factor-beta, fibroblast growth factor, and bone morphogenetic proteins. The author isolated an **amino-terminal fragment** (**ATF**) of the protease **urokinase** (uPA) from the conditioned medium of the prostate carcinoma cell line PC-3 and demonstrated that this fragment has mitogenic activity for osteoblastic cells. The activity appears to reside in an epidermal growth factor-like growth factor domain (GFD) within the **ATF**. Subsequently, the author cloned the rat uPA receptor (uPAR). uPAR is known to bind the **ATF** and can permit the uPA molecule to exhibit focal proteolysis. It was shown that the **ATF** also can induce c-myc, c-jun, and c-fos in osteoblastic cells. This effect of **ATF** can be mimicked by the GFD and suggests that this signalling pathway in osteoblasts is via the uPAR. Consequently, the uPA molecule may contribute to growth factor effects in osteoblasts via the NH2-terminal fragment and to tumor invasiveness via its COOH-terminal proteolytic domain. This scenario is supported by results from studies with uPA-overexpressing prostate carcinoma cells in rats. Additional studies will be required to further define the mechanisms of interaction of prostate carcinoma and other cancers with bone but each site of molecular interaction may provide a therapeutic window for curtailing the effects of these tumors on the skeleton.

97415430. PubMed ID: 9271229. Defective cell migration in an ovarian cancer cell line is associated with impaired **urokinase**-induced tyrosine phosphorylation. Mirshahi S S; Lounes K C; Lu H; Pujade-Lauraine E; Mishal Z; Benard J; Bernadou A; Soria C; Soria J. (Laboratoire Sainte Marie, Hotel Dieu, Parvis de Notre Dame, Paris, France.) FEBS letters, (1997 Jul 14) 411 (2-3) 322-6. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB The **urokinase** receptor (u-PAR), a protein anchored to cell membrane by a glycosyl phosphatidylinositol, plays a central role in cancer cell invasion and metastasis by binding **urokinase** plasminogen activator (u-PA), thereby facilitating plasminogen activation. Plasmin can promote cell migration either directly or by activating metalloproteinases that degrade some of the components of the extra cellular matrix. However, the IGR-OV1-Adria cell line contains the u-PAR but does not migrate even in the presence of exogenous u-PA, although the parental IGR-OV1 cell line migrates normally in the presence of u-PA. We therefore investigated the role of cell signalling for u-PA induced cell locomotion. We show that cell migration induced by u-PA-u-PAR complex is always associated with tyrosine kinase activation for the following reasons: (1) the blockade of the u-PAR by a chimeric molecule (albumin-**ATF**) inhibits not only the u-PA-induced cell migration, but also the signalling in IGR-OV1 line; (2) the binding of u-PA to u-PAR on non-migrating IGR-OV1-Adria cells was not associated with tyrosine kinase activation; (3) the inhibition of tyrosine kinase also blocked cell migration of IGR-OV1. Therefore tyrosine kinase activation seems to be essential for the u-PA-induced cell locomotion possibly by the formation of a complex u-PAR-u-PA with a protein whose transmembrane domain can ensure cell signalling. Thus, IGR-OV1 and IGR-OV1-Adria cell lines represent a good model for the analysis of the mechanism of u-PA-u-PAR-induced cell locomotion.

L15 ANSWER 62 OF 129 MEDLINE on STN

97402957. PubMed ID: 9258335. Induction in human osteoblastic cells (SaOS2) of the early response genes fos, jun, and myc by the **amino terminal fragment** (**ATF**) of **urokinase**. Rabbani S A; Gladu J; Mazar A P; Henkin J; Goltzman D. (Department of Medicine, McGill University, Montreal, Quebec, Canada.) Journal of cellular physiology, (1997 Aug) 172 (2) 137-45. Journal code: 0050222. ISSN: 0021-9541. Pub. country: United States. Language: English.

AB Previous studies have demonstrated that overexpression of urinary plasminogen activator (uPA) in rat prostate cancer cells results in increased skeletal metastases, which are primarily of the osteoblastic variety. The osseous activation induced by the metastases appears to be mediated through the **amino terminal fragment** (**ATF**) of uPA, which lacks the catalytic domain and can act as a growth factor for osteoblasts. To explore further the mechanism of action of uPA in bone cells, we evaluated the effects of **ATF** on modulating the expression of various proto-oncogenes. Human-osteoblast-derived osteosarcoma cells, SaOS2, were treated with graded doses of **ATF** for 10-120 min, and effects on early response proto-oncogenes were monitored. **ATF** increased c-myc, c-jun, and c-fos gene expression in a time-dependent manner for up to 60 min, after which mRNA levels fell. The maximum induction was seen in c-fos gene expression, which was found to be dose dependent. This effect of **ATF** was localized to its growth-factorlike domain. Examination of the half life of these transcripts in the presence of the transcriptional inhibitor actinomycin D demonstrated that **ATF** does not alter the stability of c-fos mRNA in these bone cells. Nuclear run-off assays indicated that **ATF** effects were due to stimulation of c-fos gene transcription. An increase in c-fos protein levels was correlated with the augmentation of its mRNA in **ATF**-treated SaOS2 cells. Pretreatment of SaOS2 cells with the protein tyrosine kinase inhibitor herbimycin and recombinant soluble uPA receptor (uPAR) caused a significant reduction in the ability of **ATF** to induce c-fos expression. These results demonstrate a novel role for uPA in activating early response proto-oncogenes, in particular c-fos, which plays an important role in bone cell growth and differentiation and may be a key factor in the signal

transduction pathway of **ATF**.

L15 ANSWER 63 OF 129 MEDLINE on STN

97318767. PubMed ID: 9175704. Evidence of a non-conventional role for the **urokinase** tripartite complex (uPAR/uPA/PAI-1) in myogenic cell fusion. Bonavaud S; Charriere-Bertrand C; Rey C; Leibovitch M P; Pedersen N; Frisdal E; Planus E; Blasi F; Gherardi R; Barlovatz-Meimon G. (Groupe d'Etudes et de Recherches sur le Muscle et le Nerf (GERMEN: ER 269+ 315), Universite Paris XII, Creteil, France.) Journal of cell science, (1997 May) 110 (Pt 9) 1083-9. Journal code: 0052457. ISSN: 0021-9533. Pub. country: ENGLAND: United Kingdom. Language: English.

AB **Urokinase** can form a tripartite complex binding **urokinase** receptor (uPAR) and plasminogen activator inhibitor type-1 (PAI-1), a component of the extracellular matrix (ECM). The components of the tripartite complex are modulated throughout the in vitro myogenic differentiation process. A series of experiments aimed at elucidating the role of the **urokinase** tripartite complex in the fusion of human myogenic cells were performed in vitro. Myogenic cell fusion was associated with increased cell-associated **urokinase**-type plasminogen activator (uPA) activity, cell-associated uPAR, and uPAR occupancy. Incubation of cultures with either uPA anticatalytic antibodies, or the **amino-terminal fragment** of uPA (**ATF**), which inhibits competitively uPA binding to its receptor, or anti-PAI-1 antibodies, which inhibit uPA binding to PAI-1, resulted in a 30 to 47% decrease in fusion. Incubation of cultures with the plasmin inhibitor aprotinin did not affect fusion. Decreased fusion rates induced by interfering with uPAR/uPA/PAI-1 interactions were not associated with significant changes in mRNA levels of both the myogenic regulatory factor myogenin and its inhibitor of DNA binding, Id. Incubation of cultures with purified uPA resulted in a decrease in fusion, likely due to a competitive inhibition of PAI-1 binding of endogenous uPA. We conclude that muscle cell fusion largely depends on interactions between the members of the **urokinase** complex (uPAR/uPA/PAI-1), but does not require proteolytic activation of plasmin. Since the intrinsic muscle cell differentiation program appears poorly affected by the state of integrity of the **urokinase** complex, and since cell migration is a prerequisite for muscle cell fusion in vitro, it is likely that the **urokinase** system is instrumental in fusion through its connection with the cell migration process. Our results suggest that the **urokinase** tripartite complex may be involved in cell migration in a non conventional way, playing the role of an adhesion system bridging cell membrane to ECM.

L15 ANSWER 64 OF 129 MEDLINE on STN

97286953. PubMed ID: 9142045. Beta 2 (CD11/CD18) integrins can serve as signaling partners for other leukocyte receptors. Todd R F 3rd; Petty H R. (Department of Internal Medicine, University of Michigan Medical School, Ann Arbor 48109-0374, USA.) Journal of laboratory and clinical medicine, (1997 May) 129 (5) 492-8. Ref: 64. Journal code: 0375375. ISSN: 0022-2143. Pub. country: United States. Language: English.

AB Fig. 1 depicts our current thinking about the ways in which Mo1 and p150,95 form cis interactions with other leukocyte receptors. With respect to the associations of Mo1 with Fc gamma RIIIB and uPAR, the inhibitory effect of saccharides such as NADG suggests a lectin-carbohydrate interaction that may involve the recognition of Mo1's beta-glucan site for N-linked carbohydrates4 that are expressed by both Fc gamma RIIIB and uPAR. This hypothesis is supported by the results of Stockl et al., who showed that the binding of C-terminal-specific mAb VIM12 to Mo1, which enhances the phospholipase C-mediated release of Fc gamma RIIIB, was inhibited by NADG. However, unlike the sample lectin-carbohydrate interaction that appears to govern the association between Mo1 and Fc gamma RIIIB, effective Mo1-dependent uPAR signaling also depends on the binding of intact uPA to uPAR (the receptor-binding **ATF** of uPA proving insufficient to prime neutrophils for an enhanced burst response to FMLP). We speculate that **ATF** (residues 6-135) binds to uPAR while the carboxyl terminal fragment (residues 136-411), which includes a glycosylation site at residue 144, binds to the lectinlike site of Mo1, thus fostering the linkage between the two receptors. In support

of this model is the fact that exposure of neutrophils to **ATF** reduced the degree of molecular proximity between Mo1 and uPAR (the latter probably occupied by endogenous intact uPA) and increased the molecular association between Mo1 and Fc gamma RIIIB (both as detected by quantitative RET). This hypothesis is analogous to the concept proposed by Nykjaer et al in which plasminogen activator inhibitor-1 initially binds to uPA to form a complex that secondarily binds to the alpha 2 macroglobulin receptor, leading to internalization of the complex. Whereas the contribution of intact uPA to the interaction between Mo1 and uPAR remains speculative (based on the indirect data available), no such ambiguity exists for the role of the LPS/LBP ligand in regulating the association between Mo1 and CD14. In this circumstance, no physical linkage exists between the two receptors without the ligand complex. This observation is consistent with the previously described affinity of the beta 2 integrins for LPS, leading to the notion that the LPS portion of the LPS/LPB complex binds to Mo1, serving to link it with LPS/LBP bound to CD14. The observed reversibility of the interactions between the integrin glycoproteins and uPAR or CD14 illustrates the fact that these associations can be highly dynamic and tied to cellular processes that include directed motility (Mo1-uPAR), adherence to substrates (Mo1-CD14), and energy metabolism (p150,95-uPAR). We speculate that the GPI-anchored receptor proteins serve as rapidly diffusible, expendable "scouts" for the beta 2 integrins, which serve to expand their ligand binding repertoire in a cis-acting fashion.

L15 ANSWER 65 OF 129 MEDLINE on STN

97250991. PubMed ID: 9096674. Melanoma cell migration on vitronectin: regulation by components of the plasminogen activation system. Stahl A; Mueller B M. (Department of Immunology, Scripps Research Institute, La Jolla, CA 92037, USA.) International journal of cancer. Journal international du cancer, (1997 Mar 28) 71 (1) 116-22. Journal code: 0042124. ISSN: 0020-7136. Pub. country: United States. Language: English.

AB Tumor cell migration and invasion require complex interactions between tumor cells and the surrounding extracellular matrix. These interactions are modified by cell adhesion receptors, as well as by proteolytic enzymes and their receptors. Here, we study the influence of the protease urokinasetype plasminogen activator (uPA) and its receptor (uPAR) on melanoma cell adhesion to, and migration on, the extracellular matrix protein vitronectin (VN). Cell adhesion to VN, but not to type I collagen, is significantly enhanced in the presence of either uPA or its **amino-terminal fragment** (**ATF**). Soluble uPAR can inhibit this effect, indicating that uPA/uPAR on melanoma cells can function as a VN receptor. In the absence of bivalent cations, uPA/uPAR can promote cell attachment on VN, but not cell spreading, suggesting that the glycosylphosphatidylinositol (GPI)-anchored uPAR alone is unable to organize the cytoskeleton. Chemotactic melanoma cell migration on a uniform VN matrix is inhibited by uPA and **ATF**, implying that cell motility decreases when uPA/uPAR acts as a VN receptor. In contrast, plasminogen activator inhibitor I (PAI-I) can stimulate melanoma cell migration on VN, presumably by inhibiting uPA/uPAR-mediated cell adhesion to VN and thereby releasing the inhibition of cell migration induced by uPA. Together, our data implicate components of the plasminogen activation system in the direct regulation of cell adhesion and migration, thereby modulating the behavior of malignant tumor cells.

L15 ANSWER 66 OF 129 MEDLINE on STN

97228369. PubMed ID: 9117184. Bone matrix degradation by the plasminogen activation system. Possible mechanism of bone destruction in arthritis. Ronday H K; Smits H H; Quax P H; van der Pluijm G; Lowik C W; Breedveld F C; Verheijen J H. (Department of Vascular and Connective Tissue Research, Gaubius Laboratory, TNO-PG, Leiden, The Netherlands.) British journal of rheumatology, (1997 Jan) 36 (1) 9-15. Journal code: 8302415. ISSN: 0263-7103. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The observed increase in **urokinase**-type plasminogen activator (u-PA) and its receptor (u-PAR) in synovial tissue of patients with rheumatoid arthritis (RA) suggests pathophysiological involvement of the plasminogen

activation (PA) system in inflammatory joint disease. In the present study, we investigated the capacity of the PA system to degrade non-mineralized and mineralized bone-like matrix in vitro as a model for bone destruction. Transfected mouse LB6 cell lines, that expressed either human u-PA or u-PAR, were cultured separately and simultaneously on radiolabelled bone matrix in the presence of plasminogen. Osteoblast-like murine calvarial MC3T3-E1 cells were used to produce a well-characterized, highly organized bone-like matrix, that could be mineralized in the presence of beta-glycerol phosphate. Bone matrix degradation was followed by the release of radioactivity in the culture medium. u-PA-producing cells, in contrast to u-PAR-producing cells, degraded both non-mineralized and mineralized bone matrix. This effect could be inhibited by anti-u-PA antibodies, as well as by tranexamic acid and by aprotinin, indicating that the degrading activity is u-PA mediated and plasmin dependent. Co-cultivation of a small portion of u-PA-producing cells with u-PAR-expressing cells resulted in a marked increase in degradation activity. Reduction of this potentiating effect by suramin or the **amino-terminal fragment** of u-PA, both competitive inhibitors of u-PA receptor binding, shows that this synergistic effect is due to binding of u-PA to u-PAR. u-PAR must be cell associated, as binding of u-PA to a soluble u-PAR prevented this enhancement. The capability of the PA system to degrade bone matrix in vitro, and the previously demonstrated increased expression of u-PA and u-PAR in synovial tissue of patients with RA, further support a role for the PA system in the development of bone erosions.

L15 ANSWER 67 OF 129 MEDLINE on STN

97228079. PubMed ID: 9115983. Interaction of **urokinase**-type plasminogen activator with its receptor rapidly induces activation of glucose transporters. Anichini E; Zamperini A; Chevanne M; Caldini R; Pucci M; Fibbi G; Del Rosso M. (Istituto di Patologia Generale, Universita di Firenze, Italy.) Biochemistry, (1997 Mar 18) 36 (11) 3076-83. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB The interaction of **urokinase**-type plasminogen activator (u-PA) or of u-PA **amino-terminal fragment** (u-PA-**ATF**) with the cell surface receptor (u-PAR) was found to stimulate an increase of glucose uptake in many cell lines, ranging from normal and transformed human fibroblasts, mouse fibroblasts transfected with human u-PAR, and cells of epidermal origin. Such increase of glucose uptake reached a peak within 5-10 min, depending on the cell line, and occurred through the facilitative glucose transporters (GLUTs), since it was inhibited by cytochalasin B. Each cell line showed a specific mosaic of glucose transporter isoforms, GLUT2 being the most widespread and GLUT1 the most abundant, when present. u-PAR stimulation was followed by translocation of GLUT1 from the microsomal to the membrane compartment, as shown by both immunoblotting and immunofluorescence of sonicated plasma membrane sheets and by activation of GLUT2 on the cell surface. Both translocation and activation resulted inhibitable by protein-tyrosine kinase inhibitors and independent of downregulation of protein kinase C (PKC). The increase of intracellular glucose was followed by neosynthesis of diacylglycerol (DAG) from glucose, as previously shown. Such neosynthesis was completely inhibited by impairment of facilitative GLUT transport by cytochalasin B. DAG neosynthesis was followed by activation of PKC, whose activity translocated into the intracellular compartment (PKM), where it probably phosphorylates substrates required for u-PAR-dependent chemotaxis. Our data show that u-PAR-mediated signal transduction, related with u-PA-induced chemotaxis, involves activation of tyrosine kinase-dependent glucose transporters, leading to increased de novo DAG synthesis from glucose, eventually resulting in activation of PKC.

L15 ANSWER 68 OF 129 MEDLINE on STN

97218571. PubMed ID: 9066008. The **urokinase**-receptor (CD87) is expressed in cells of the megakaryoblastic lineage. Wohn K D; Kanse S M; Deutsch V; Schmidt T; Eldor A; Preissner K T. (Haemostasis Research Unit, Kerckhoff-Klinik, MPI, Bad Nauheim, Germany.) Thrombosis and haemostasis,

(1997 Mar) 77 (3) 540 7. Journal code: 7006003. ISSN: 0340 6245. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Megakaryocytopoiesis is governed in the bone marrow microenvironment by cellular interactions that include various adhesion receptor systems and pericellular proteolysis for proper regulation of cell motility and differentiation. In order to define the role of cell surface molecules required for these processes, we searched for protease receptors on these cells. In an in vitro system utilizing different cell lines of the megakaryoblastic lineage (MEG-01, Dami), low level surface expression of the **urokinase** (uPA) receptor was noted. Following stimulation with phorbolester (PMA), a 3-6 fold higher expression of uPA receptor over a period of up to 5 days could be observed by fluorescent activated cell-sorting as well as by direct ligand-binding of **amino-terminal fragment** of uPA or vitronectin. Together with elevated expression of alpha IIb beta 3-integrin (glycoprotein IIb/IIIa complex), double immuno-fluorescence staining of stimulated cells confirmed the increased cell surface localization of uPA receptor. Semi-quantitative RT-PCR, ligand blot analysis and measurement of cell-bound proteolytic activity revealed a differentiation-dependent upregulation of the uPA receptor expression in megakaryoblastic cell lines as in monocytic cells. Due to its glycolipid anchorage, incubation with phosphatidylinositol-specific phospholipase C reduced uPA receptor-mediated ligand binding by about 60%, uPA receptor mRNA was expressed in cultured megakaryocytes derived from bone marrow, whereas no uPA receptor mRNA was detectable in platelets. These results indicate a differentiation-dependent increase in the expression of uPA receptor in megakaryoblastic cells. The characteristics of surface expression and functionality of the receptor on megakaryocytic cells may influence their maturation by regulating cellular communication in the bone marrow micro-environment.

L15 ANSWER 69 OF 129 MEDLINE on STN

97185974. PubMed ID: 9033655. Plasminogen activators play an essential role in extracellular-matrix invasion by lymphoblastic T cells. Reiter L S; Spertini O; Kruithof E K. (Department of Medicine, University Hospital Lausanne, Switzerland.) International journal of cancer. Journal international du cancer, (1997 Feb 7) 70 (4) 461-6. Journal code: 0042124. ISSN: 0020-7136. Pub. country: United States. Language: English.

AB Involvement of extravascular sites, in particular infiltration of the central nervous system, is a frequent complication of T-lymphoblastic leukemia and contributes to leukemia-associated morbidity. In this report, we studied the contribution of plasminogen activators to the invasive properties of 7 human T-cell lines in a model of transmigration through an extracellular matrix. The T-cell lines were found to express either **urokinase** (u-PA) and high levels of u-PA receptor or tissue-type plasminogen activator (t-PA) and low levels of u-PA receptor. The rate of transmigration was consistently higher for u-PA-expressing cells than for t-PA-expressing cells. PA-inhibitor type 1 (PAI-1) was detected in the conditioned medium of one cell line and PAI-2 was detected in cell extracts from 6 lines. The transmigration of 6 out of 7 cell lines was inhibited by trasylol, an inhibitor of plasmin, by an excess of exogenous PAI-1 or PAI-2, and by antibodies to the particular PA type expressed by the cells. Partial inhibition of transmigration by the **amino-terminal fragment** of u-PA implies that the u-PA receptor contributes to transmigration. Thus, the transmigration of T-leukemia cells through a barrier of extracellular matrix requires PA-dependent proteolysis, which can be provided either by u-PA or t-PA. Specific inhibition of the PA system could provide a means to inhibit tissue invasion by T lymphoblastic cells.

L15 ANSWER 70 OF 129 MEDLINE on STN

97184198. PubMed ID: 9030610. Soluble human **urokinase** receptor is composed of two active units. Higazi A A; Mazar A; Wang J; Quan N; Griffin R; Reilly R; Henkin J; Cines D B. (Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA.) Journal of biological chemistry, (1997 Feb 21) 272 (8) 5348-53. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States.

Language: English.

AB The mechanism by which single-chain **urokinase** (scuPA) binds to its receptor (uPAR) is incompletely understood. We report that a fragment comprising the first domain of recombinant soluble uPAR (sDI) as well as a fragment comprising the remaining domains (sDII-DIII) competes with the binding of recombinant full-length soluble uPAR (suPAR) to scuPA with an IC50 = 253 nM and an IC50 = 1569, respectively. sDII-III binds directly to scuPA with Kd = 238 nM. Binding of scuPA to each fragment also induces the expression of plasminogen activator activity. sDI and sDII-DIII (200 nM each) induced activity equal to 66 and 36% of the maximum activity induced by full-length suPAR (5 nM), respectively. Each fragment also stimulates the binding of scuPA to cells lacking endogenous uPAR. Although scuPA binds to sDI and to sDII-DIII through its **amino-terminal fragment**, the fragments act synergistically to inhibit the binding of suPAR and to stimulate plasminogen activator activity. Furthermore, sDII-DIII retards the velocity and alters the pattern of cleavage of sDI by chymotrypsin. These results suggest that binding of scuPA to more than one epitope in suPAR is required for its optimal activation and association with cell membranes.

L15 ANSWER 71 OF 129 MEDLINE on STN

97022742. PubMed ID: 8869102. Immunoassays (ELISA) of **urokinase**-type plasminogen activator (uPA): report of an EORTC/BIOMED-1 workshop. Benraad T J; Geurts-Moespot J; Grondahl-Hansen J; Schmitt M; Heuvel J J; de Witte J H; Foekens J A; Leake R E; Brunner N; Sweep C G. (532 Department of Experimental and Chemical Endocrinology, University of Nijmegen, Netherlands.) European journal of cancer (Oxford, England : 1990), (1996 Jul) 32A (8) 1371-81. Ref: 28. Journal code: 9005373. ISSN: 0959-8049. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The **urokinase**-type plasminogen activator (uPA) is considered to play a key role in the process of invasion and metastasis. In several independent studies, in a variety of cancer types (e.g. of the breast, colon, stomach, lung, ovary), high antigen levels of uPA in tumour extracts have been associated with rapid disease progression. In these studies, different sets of antibodies and standards (often as commercially available uPA ELISA kits) have been used. The standards provided with the different uPA ELISA kits are different from each other in both composition and source. In addition, the different uPA ELISA kits use antibodies which differ in specificity and affinity for the various forms of uPA including pro-uPA, HMW-uPA, LMW-uPA, the aminoterminal fragment (**ATF**) and complexes with inhibitors (PAI-1 and PAI-2) and the receptor (uPAR). Further, the composition of tumour tissue extraction buffers differ significantly among the published studies. Thus, it is not surprising that the ranges of cytosolic uPA levels reported differ considerably even when measured within the same tumour type. These discrepancies led the EORTC Receptor and Biomarker Study Group, in conjunction with the BIOMED-1 consortium on 'Clinical Relevance of Proteases in Tumour Invasion and Metastasis', to organise a workshop to study the characteristics associated with six different uPA immunoassays (ELISA) used in clinical studies reported in the literature. Although the absolute uPA antigen values measured with the respective uPA ELISA kits differed, high correlations were obtained for any two of the four uPA ELISA kits finally applied to sets of breast cancer cytosol preparations. The preparations used at present as standards in the various uPA ELISA kits are not representative of actual human breast cancer cytosols. Thus absolute standardisation is only possible by using a common reference sample (breast cancer cytosol) and similarly composed ELISA uPA kits. Then it will be possible to generate comparable data on clinical tissue as well as to check for batch-to-batch variations within particular ELISA kits.

L15 ANSWER 72 OF 129 MEDLINE on STN

97010891. PubMed ID: 8857924. Contrasting effects of plasminogen activators, **urokinase** receptor, and LDL receptor-related protein on smooth muscle cell migration and invasion. Okada S S; Grobmyer S R; Barnathan E S. (University of Pennsylvania School of Medicine, Philadelphia 19104-6060, USA.) Arteriosclerosis, thrombosis, and vascular

Biology, (1996 Oct) 16 (10) 1269-76. Journal code: 9505609. ISSN: 1079-5642. Pub. country: United States. Language: English.

AB Smooth muscle cell (SMC) migration is an early response to vascular injury and contributes to the development of intimal thickening. Upregulation of several components of the plasminogen activator (PA) system has been documented after vascular injury. Utilizing a Transwell filter assay system and human umbilical vein SMCs, we sought to define the role of four different PA system components on SMC migration and matrix invasion: (1) PAs, (2) plasmin, (3) PA receptors, and (4) PA clearance receptors (ie, low density lipoprotein receptor-related protein [LRP]). Addition of active two-chain **urokinase**-type PA (UPA) stimulated random migration (192 +/- 30% of control, 0.36 nmol/L, P < .001). The stimulation was inhibited by pretreatment with diisopropylfluorophosphate, PA inhibitor type 1 (PAI-1), or aprotinin, a plasmin inhibitor. Augmented migration was also observed with either low-molecular-weight UPA or the **amino terminal fragment** of UPA (**ATF**), with the effects being additive. Stimulation by **ATF** alone, however, was not inhibited by aprotinin. The stimulatory effect was not specific for UPA, in that tissue-type PA (TPA) also increased migration (169 +/- 9% of control, 10 nmol/L, P < .001); the augmentation was inhibited by pretreatment with DFP, PAI-1, or aprotinin and was additive to the UPA effect. Antibodies to the UPA receptor but not 5'-nucleotidase (another glycosylphosphatidylinositol-anchored cell surface protein) inhibited baseline and UPA-stimulated migration. Similarly, both UPA and TPA stimulated invasion of a collagen gel; this augmentation was inhibited by aprotinin, whereas antibodies to the UPA receptor reduced baseline invasion. Finally, we tested whether inhibition of LRP function, which mediates internalization of PA/inhibitor complexes, affected either process. Both antibodies to LRP and recombinant receptor associated protein, a known inhibitor of ligand binding to the LRP, significantly inhibited migration but did not affect collagen gel invasion. These data demonstrate the ability of several components of the PA system to modulate SMC migration and invasion in vitro via plasmin-dependent and -independent mechanisms.

L15 ANSWER 73 OF 129 MEDLINE on STN

96404689. PubMed ID: 8808830. Mechanism of tumor cell-induced extracellular matrix degradation--inhibition of cell-surface proteolytic activity might have a therapeutic effect on tumor cell invasion and metastasis. Kobayashi H. (Department of Obstetrics and Gynecology, Hamamatsu University School of Medicine.) Nippon Sanka Fujinka Gakkai zasshi, (1996 Aug) 48 (8) 623-32. Ref: 14. Journal code: 7505749. ISSN: 0300-9165. Pub. country: Japan. Language: Japanese.

AB Tumor cells produce **urokinase**-type plasminogen activator (uPA) in an enzymatically inactive proenzyme form (pro-uPA). Secreted pro-uPA can immediately bind to the specific uPA receptors (uPAR) on tumor cell surface with high affinity. The uPAR specifically recognizes enzymatically inactive pro-uPA and active high molecular weight-uPA (HMW-uPA) by their growth factor-like terminal domain. uPAR is a glycoprotein of approximately 55 kDa; the affinity for uPA is high (0.2 nM) and the rate of dissociation is low. Receptor-bound uPA catalizes the formation of plasmin on the cell surface to generate the proteolytic cascade that contributes to the breakdown of basement membrane and extracellular matrix. The plasma membrane uPAR has attracted considerable attention because of its role in migration and tissue invasion by mononuclear phagocytes and malignant cells. In some cell types uPAR localizes uPA to cell-cell and cell-substratum contact sites, providing the possibility of a directional proteolysis that may be involved in cell migration and invasion. Recently it has been reported that competitive displacement of uPA from uPAR resulted in decreased proteolysis, suggesting that the cell surface is the preferred site for uPA-mediated protein degradation. Various very different approaches to interfere with the expression or reactivity of uPA or uPAR at the gene or protein level were successfully tested including antisense oligonucleotides, antibodies, inhibitors and recombinant or synthetic uPA and uPAR analogues. Recently we have reported that a highly purified urinary trypsin inhibitor (UTI) efficiently inhibits soluble and tumor cell-surface receptor-bound

plasmin. UTI inhibits not only tumor cell invasion in an in vitro assay but also production of experimental and spontaneous lung metastasis in an in vivo mouse model. The anti-invasive effect is dependent on the anti-plasmin activity of UTI. UTI peptide, which inhibits plasmin activity, synthesized by an automated peptide synthesizer showed mouse 3LL cell invasion inhibitory activity. UTI and the effective peptide inhibited tumor cell invasion through Matrigel. UTI did not inhibit tumor cell proliferation or the binding of the cells to Matrigel. Also, UTI did not inhibit chemotactic migration of tumor cells to fibronectin. It is likely that UTI acts as a protease inhibitor. We attempted to synthesize conjugates between **ATF** and UTI. Thus, conjugating a physiological plasmin inhibitor to **ATF** might target it to reduce cell-associated proteolytic activity to the close environment of the uPAR-expressing tumor cell surface and subsequently may effectively inhibit tumor cell invasion and metastasis, because the cell surface uPAR might be a critical component of the metastatic machinery. A method of conjugation of the UTI domain II (HI-8), to the receptor-binding **amino-terminal fragment** (**ATF**) of uPA has been developed utilizing the heterobifunctional cross-linking reagent, N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP). The conjugate retained its protease inhibiting activity and showed a binding reactivity to uPAR on the surface of tumor cells. We have shown that the conjugate exhibits plasmin inhibition to the close environment of the cell surface and subsequently inhibits the tumor cell invasion through Matrigel in an in vitro invasion assay. In order to extend our idea, we attempt to produce a novel hybrid molecule consisting of the **ATF** of uPA placed at the N-terminus of UTI domain II (HI-8) by protein engineering techniques. Exogenously applied ATFHI hybrid protein can immediately bind to the specific uPAR on cell surfaces with high affinity. The receptor-bound hybrid protein focuses the protease-inhibiting activity to the tumor cell surface. This is effectively a bifunctional molecule which, in addition to inhibiting trypsin and plasmin activities directly, is able toblock unoccupied uPAR, thereby preventing localization of uPA activity.

L15 ANSWER 74 OF 129 MEDLINE on STN

96394362. PubMed ID: 8798468. Domain interplay in the **urokinase** receptor. Requirement for the third domain in high affinity ligand binding and demonstration of ligand contact sites in distinct receptor domains. Behrendt N; Ronne E; Dano K. (Finsen Laboratory, Rigshospitalet, Strandboulevarden 49, Building 7. 2, DK-2100 Copenhagen O, Denmark.) Journal of biological chemistry, (1996 Sep 13) 271 (37) 22885-94. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The **urokinase** plasminogen activator receptor (uPAR) is a membrane protein comprised of three extracellular domains. In order to study the importance of this domain organization in the ligand-binding process of the receptor we subjected a recombinant, soluble uPAR (suPAR) to specific proteolytic cleavages leading to liberation of single domains. Treatment of the receptor with pepsin resulted in cleavage between residues 183 and 184, thus separating the third domain (D3) from the rest of the molecule, which was left as an intact fragment (D(1 + 2)). D(1 + 2) proved capable of ligand binding as shown by chemical cross-linking, but quantitative binding/competition studies showed that the apparent ligand affinity was 100- to 1000-fold lower than that of the intact suPAR. This loss of affinity was comparable with the loss found after cleavage between the first domain (D1) and D(2 + 3), using chymotrypsin. This result shows that in addition to D1, which has an established function in ligand binding (Behrendt, N., Ploug, M., Patthy, L., Houen, G., Blasi, F., and Dano, K. (1991) J. Biol. Chem. 266, 7842-7847), D3 has an important role in governing a high affinity in the intact receptor. Real-time biomolecular interaction analysis revealed that the decrease in affinity was caused mostly by an increased dissociation rate of the ligand complex of D(1 + 2). Zero length cross-linking, using carbodiimide-induced, direct condensation, was used to identify regions within suPAR engaged in molecular ligand contact. The purified suPAR was cross-linked to the radiolabeled **amino-terminal fragment** (**ATF**) of **urokinase**,

followed by cleavage with chymotrypsin. In accordance with the cleavage pattern found for the uncomplexed receptor, this treatment led to cleavage between D1 and D(2 + 3). Analysis of the radiolabeled fragments revealed the expected ligand labeling of D1 but a clear labeling of D(2 + 3) was also found, indicating that this part of the molecule is also situated in close contact with **ATF** in the receptor-ligand complex. The latter contact site may contribute to the role of molecular regions outside D1 in high affinity binding.

L15 ANSWER 75 OF 129 MEDLINE on STN

96273549. PubMed ID: 8695276. In vitro anti-proliferative and anti-invasive role of aminoterminal fragment of **urokinase**-type plasminogen activator on 8701-BC breast cancer cells. Luparello C; Del Rosso M. (Dipartimento di Biologia Cellulare e dello Sviluppo, Universita, Palermo, Italy.) European journal of cancer (Oxford, England : 1990), (1996 Apr) 32A (4) 702-7. Journal code: 9005373. ISSN: 0959-8049. Pub. country: ENGLAND: United Kingdom. Language: English.

AB 8701-BC cells, derived from a primary carcinoma of the breast, constitutively express mRNA for **urokinase**-type plasminogen activator (uPA). In this paper, we demonstrated the presence of uPA in the conditioned medium, and of uPA-receptor (uPAR) on the cell surface of 8701-BC cells, which therefore have the potential for an autocrine mechanism of uPA-mediated stimulation. We examined whether exogenous addition of either intact uPA, or its **amino-terminal fragment** (uPA-**ATF**), which lacks catalytic activity but retains the uPAR binding site and a growth factor-like domain, or immunoneutralisation of endogenous uPA-uPAR interactions could exert any effect on the proliferative and invasive behaviour of 8701-BC cells. The data demonstrate that, while uPA promotes growth and invasion of 8701-BC cells, its effect reversed by blocking uPA-uPAR interactions, uPA-**ATF** not only fails to impart growth factor-like signals, but also restrains cell invasion in vitro. In the light of these and other data, an active participation of **ATF** in the complex cell-ECM network of interactions underlying cancer progression can be postulated. In addition, it appears worth considering the possibility of testing the effect of this uPA fragment in vivo for the therapy of breast (and possibly other) human invasive carcinomas.

L15 ANSWER 76 OF 129 MEDLINE on STN

96235241. PubMed ID: 8647121. Systematic mutational analysis of the receptor-binding region of the human **urokinase**-type plasminogen activator. Magdolen V; Rettenberger P; Koppitz M; Goretzki L; Kessler H; Weidle U H; Konig B; Graeff H; Schmitt M; Wilhelm O. (Frauenklinik der Technischen Universitat Munchen, Germany.) European journal of biochemistry / FEBS, (1996 May 1) 237 (3) 743-51. Journal code: 0107600. ISSN: 0014-2956. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB The **amino-terminal fragment** of human uPA (**ATF**; amino acids 1-135), which contains the binding site for the uPA receptor (uPAR, CD87) was expressed in the yeast Saccharomyces cerevisiae. Recombinant yeast **ATF**, modified and extended by an amino-terminal in-frame insertion of a His6 tract, was purified from total protein extracts by nickel chelate affinity chromatography and shown to be functionally active since it efficiently competes with uPA for binding to cell-surface-associated uPAR. The **ATF** expression plasmid served as a template for the construction of a series of site-directed mutants in order to define those amino acids that are important for binding to uPAR. All mutant **ATF** proteins but one (deletion of Ser26) were expressed in a stable form (about 20-30 ng/mg total protein) and the binding capacity of each mutant was tested by a uPA-ligand binding assay employing recombinant uPAR immobilized to a microtiter plate. Each of the 11 amino acids of loop B of the binding region of uPA (amino acids 20-30) were individually substituted with alanine. Lys23, Tyr24, Phe25, IIe28, and Trp30 were important determinants for uPAR binding. A systematic alanine scan was also performed with chemically synthesized linear peptides spanning amino acids 14-32 of **ATF**. Comparable results to those with the yeast **ATF** mutants

were obtained. In a different set of experiments, those amino acids of the uPAR-binding region of uPA that are only conserved between man and baboon but not in other species were altered: whereas substitution of Thr18 by alanine or Asn32 by serine had hardly any effect, replacement of Asn22 by tyrosine and Trp30 by arginine (both positions are strictly conserved in other mammals) led to **ATF** variants incapable of interacting with human uPAR. Deletion of either Val20, Ser21, Lys23, His29 or Val20 plus Ser21, respectively, also generated non-reactive **ATF** mutants. Finally, Lys23 in **ATF** was substituted with certain amino acids: whereas the replacement of Lys23 by alanine, histidine or glutamine generated **ATF** variants with moderate uPAR-binding activity, the introduction of a negatively charged amino acid (exchange of Lys23 by glutamic acid) completely abolished uPAR-binding activity. The results presented for the **ATF** mutants and uPA-derived peptides may provide clues necessary to establish the nature of the physical interaction of uPA with its receptor and may help to develop uPA-derived peptide analogues as potential therapeutic agents to block tumor cell-associated uPA/uPAR interaction.

L15 ANSWER 77 OF 129 MEDLINE on STN

96219970. PubMed ID: 8639894. Evidence for a novel binding protein to **urokinase**-type plasminogen activator in platelet membranes. Jiang Y; Pannell R; Liu J N; Gurewich V. (Institute for the Prevention of Cardiovascular Disease, Deaconess Hospital, Harvard Medical School, Boston, MA, USA.) Blood, (1996 Apr 1) 87 (7) 2775-81. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB Endogenous **urokinase**-type plasminogen activator (u-PA) has been identified in platelet membrane, and platelets have been shown to take up exogenous high molecular weight u-PA from the ambient medium. In this report, the mechanism of the association of u-PA with platelets was investigated using recombinant, single chain u-PA. When gel filtered human platelets were incubated with radiolabeled u-PA, the u-PA was found to specifically and saturably bind to the resting platelets in a dose-dependent manner. Unlabeled u-PA and the **amino terminal fragment** of u-PA inhibited 125I-u-PA binding to platelets with a mean IC50 of 65 and 58 nmol/L, respectively. A single saturable binding site in intact resting platelets was found with a mean kd of 43 +/- 25 nmol/L and 2263 +/- 809 sites per platelet. In contrast to resting platelets, 125I-u-PA did not bind to thrombin-induced platelets. Western blotting studies, using a monoclonal or a polyclonal antibody specific for the u-PA cell-surface receptor (u- PAR), failed to show evidence of u-PAR in resting platelets, whereas, u-PAR was found at approximately 54 and approximately 48 kD on U937 monocytes, which served as a positive control. Ligand blotting of platelet membrane and of U937 cell proteins with 125I-u-PA revealed a u-PA binding protein of approximately 70 kD in the platelets and one of approximately 54 kD in the U937 cells. Complexion of u-PA with a platelet membrane protein was also shown by gel filtration of a mixture of u-PA and platelet membrane proteins. A u-PA complex was further shown by enzyme-linked immunosorbent assay when microtiter plates were coated with platelet membrane proteins, and this complex formation was shown to be dose-dependent and saturable with an apparent kd of 17 nmol/L. It was concluded that platelet membrane contains a specific, high affinity u-PA-binding protein that is distinct from u-PAR.

L15 ANSWER 78 OF 129 MEDLINE on STN

96203075. PubMed ID: 8612581. Proteolytic cleavage of the **urokinase** receptor substitutes for the agonist-induced chemotactic effect. Resnati M; Guttinger M; Valcamonica S; Sidenius N; Blasi F; Fazioli F. (Department of Biology and Biotechnology, San Raffaele Scientific Institute, Milano, Italy.) EMBO journal, (1996 Apr 1) 15 (7) 1572-82. Journal code: 8208664. ISSN: 0261-4189. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Physiological concentrations of **urokinase** plasminogen activator (uPA) stimulated a chemotactic response in human monocytic THP-1 through binding to the **urokinase** receptor (uPAR). The effect did not require the protease moiety of uPA, as stimulation was achieved also with the N-terminal fragment (**ATF**), while the 33 kDa low molecular weight uPA was

ineffective. Co-immunoprecipitation experiments showed association of uPAR with intracellular kinase(s), as demonstrated by in vitro kinase assays. Use of specific antibodies identified p56/p59hck as a kinase associated with uPAR in THP-1 cell extracts. Upon addition of **ATF**, p56/p59hck activity was stimulated within 2 min and returned to normal after 30 min. Since uPAR lacks an intracellular domain capable of interacting with intracellular kinase, activation of p56/p59hck must require a transmembrane adaptor. Evidence for this was strongly supported by the finding that a soluble form of uPAR (suPAR) was capable of inducing chemotaxis not only in THP-1 cells but also in cells lacking endogenous uPAR (IC50, 5 pM). However, activity of suPAR require chymotrypsin cleavage between the N-terminal domain D1 and D2 + D3. Chymotrypsin-cleaved suPAR also induced activation of p56/p59hck in THP-1 cells, with a time course comparable with **ATF**. Our data show that uPA-induced signal transduction takes place via uPAR, involves activation of intracellular tyrosine kinase(s) and requires an as yet undefined adaptor capable of connecting the extracellular ligand binding uPAR to intracellular transducer(s).

L15 ANSWER 79 OF 129 MEDLINE on STN

96198033. PubMed ID: 8612711. The **urokinase** receptor is a major vitronectin-binding protein on endothelial cells. Kanse S M; Kost C; Wilhelm O G; Andreasen P A; Preissner K T. (Haemostasis Research Unit, Kerckhoff-Klinik, Bad Nauheim, Germany.) Experimental cell research, (1996 May 1) 224 (2) 344-53. Journal code: 0373226. ISSN: 0014-4827. Pub. country: United States. Language: English.

AB We have previously demonstrated that vitronectin (VN), a morphoregulatory protein in the vessel wall, is internalized and translocated to the subendothelial matrix by an integrin-independent mechanism (J. Histochem. Cytochem. 41, 1823-1832, 1993). The cell surface component which mediates the initial contact of VN with endothelial cells is defined here. The specific binding of VN to endothelial cells demonstrated the following properties: a threefold increase after phorbol ester treatment; 85% inhibition by pretreatment of cells with phosphatidylinositol-phospholipase C to release glycolipid-anchored surface proteins; a 90% inhibition by **urokinase** (u-PA) receptor blocking antibody. u-PA increased VN binding to cells due to an eightfold increase in the affinity of VN for the u-PA receptor. Structure-function studies showed that the **amino-terminal fragment** of u-PA, devoid of any proteolytic activity, mediated this effect. Active plasminogen activator inhibitor-1 (PAI-1), but not inactivated PAI-1, inhibited VN binding to cells and displaced VN that was prebound to endothelial cell monolayers. Similarly, VN binding to purified (immobilized) u-PA receptor, but not to integrin, was enhanced by u-PA and inhibited by PAI-1. Hence, the binding of soluble VN to endothelial cell surfaces is mediated by the u-PA receptor, and the relative concentrations of u-PA and PAI-1 are able to regulate the strength of this interaction. Endothelial cell adhesion to immobilized VN was found to be integrin-mediated without any involvement of the VN-uPA-receptor system. Hence, the interaction of VN with the u-PA receptor may be involved in the regulation of cellular processes necessary for endothelial cell invasion and migration at VN-rich extracellular matrix sites.

L15 ANSWER 80 OF 129 MEDLINE on STN

96193900. PubMed ID: 8641412. Removal of domain D2 or D3 of the human **urokinase** receptor does not affect ligand affinity. Riittinen L; Limongi P; Crippa M P; Conese M; Hernandez-Marrero L; Fazioli F; Blasi F. (Department of Biology and Biotechnology (DIBIT), San Raffaele Scientific Institute, Milan, Italy.) FEBS letters, (1996 Feb 26) 381 (1-2) 1-6. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB The main ligand-binding determinant of the human **urokinase** receptor (uPAR) is located in the amino terminal domain D1, but when isolated this domain presents a 1500 fold lower affinity than the intact three-domain uPAR (D1D2D3). uPAR mutants missing either domain 2 (D1HD3) or domain 3 (D1D2) were expressed in murine LB6 cells and showed to be properly

GPI anchored to the cell surface. Binding assays with [125I]**ATF** demonstrated that these mutants possessed a normal (D1D2) or slightly reduced (D1HD3) affinity, indicating that a high ligand-affinity may be achieved by a combination of D1 with domain D2 or D3.

L15 ANSWER 81 OF 129 MEDLINE on STN

96181660. PubMed ID: 8603739. Blockage of **urokinase** receptor reduces in vitro the motility and the deformability of endothelial cells. Lu H; Mabilat C; Yeh P; Guitton J D; Li H; Pouchelet M; Shoevaert D; Legrand Y; Soria J; Soria C. (INSERM U353, Hopital St. Louis, Paris, France.) FEBS letters, (1996 Feb 12) 380 (1-2) 21-4. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB The binding of **urokinase** (u-PA) to its cell surface receptor (u-PAR) is critical for tumor cell invasion. Here, we report that the distribution of this binding by a u-PAR antagonist **ATF**-HSA inhibits in vitro the motility of endothelial cells in a dose-dependent manner. This inhibition was also observed when the cells were first stimulated with potent angiogenic factors, including bFGF or VEGF. [3H]thymidine incorporation assay demonstrated that **ATF**-HSA did not affect the cell proliferation. **ATF**-HSA was more potent than plasmin inhibitors, suggesting that it exerts its effects not solely by inhibiting the remodeling of the extracellular matrix. In fact, analysis of the cell shape change during migration revealed for the first time that its effect is related to a decrease in cell deformability. These results suggest that u-PAR antagonist may be a new approach to control angiogenesis.

L15 ANSWER 82 OF 129 MEDLINE on STN

96176310. PubMed ID: 8601593. Cooperative effect of TNFalpha, bFGF, and VEGF on the formation of tubular structures of human microvascular endothelial cells in a fibrin matrix. Role of **urokinase** activity. Koolwijk P; van Erck M G; de Vree W J; Vermeer M A; Weich H A; Hanemaaijer R; van Hinsbergh V W. (Gaubius Laboratory TNO-PG, Leiden, The Netherlands.) Journal of cell biology, (1996 Mar) 132 (6) 1177-88. Journal code: 0375356. ISSN: 0021-9525. Pub. country: United States. Language: English.

AB In angiogenesis associated with tissue repair and disease, fibrin and inflammatory mediators are often involved. We have used three-dimensional fibrin matrices to investigate the humoral requirements of human microvascular endothelial cells (hMVEC) to form capillary-like tubular structures. bFGF and VEGF165 were unable to induce tubular structures by themselves. Simultaneous addition of one or both of these factors with TNFalpha induced outgrowth of tubules, the effect being the strongest when bFGF, VEGF165, and TNFalpha were added simultaneously. Exogenously added u-PA, but not its nonproteolytic **amino-terminal fragment**, could replace TNFalpha, suggesting that TNFalpha-induced u-PA synthesis was involved. Soluble u-PA receptor (u-PAR) or antibodies that inhibited u-PA activity prevented the formation of tubular structures by 59-99%. epsilon-ACA and trasylol which inhibit the formation and activity of plasmin reduced the extent of tube formation by 71-95%. TNFalpha or u-PA did not induce tubular structures without additional growth factors. bFGF and VEGF165 enhanced of the u-PAR by 72 and 46%, but TNFalpha itself also increased u-PAR in hMVEC by 30%. Induction of mitogenesis was not the major contribution of bFGF and VEGF165 because the cell number did not change significantly in the presence of TNFalpha, and tyrphostin A47, which inhibited mitosis completely, reduced the formation of tubular structures only by 28-36%. These data show that induction of cell-bound u-PA activity by the cytokine TNFalpha is required in addition to the angiogenic factors VEGF165 and/or bFGF to induce in vitro formation of capillary-like structures by hMVEC in fibrin matrices. These data may provide insight in the mechanism of angiogenesis as occurs in pathological conditions.

L15 ANSWER 83 OF 129 MEDLINE on STN

96145724. PubMed ID: 8590627. A competitive chromogenic assay to study the functional interaction of **urokinase**-type plasminogen activator with its receptor. Rettenberger P; Wilhelm O; Oi H; Weidle U H; Goretzki L; Koppitz M; Lottspeich F; Konig B; Pessara U; Kramer M D; +. (Frauenklinik,

Technischen Universitat Munchen, Germany.) Biological chemistry Hoppe-Seyler, (1995 Oct) 376 (10) 587-94. Journal code: 8503054. ISSN: 0177-3593. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB **Urokinase**-type plasminogen activator (uPA) converts plasminogen to plasmin which degrades various extracellular matrix components. uPA is focused to the cell surface via binding to a specific receptor (uPAR, also termed CD87). uPAR-bound uPA mediates pericellular proteolysis in a variety of biological processes, e.g. cell migration, tissue remodeling and tumor invasion. We have developed a competitive microtiter plate-based chromogenic assay which allows the analysis of uPA/uPAR interaction. The plates are coated with recombinant uPAR expressed in Chinese hamster ovary (CHO) cells. Proteolytically active uPA (HMW-uPA) is added to the microtiter plate-attached uPAR. The amount of receptor-bound uPA is then determined indirectly via addition of plasminogen, which is activated to plasmin, followed by cleavage of a plasmin-specific chromogenic substrate. Substances interfering with binding of HMW-uPA to uPAR diminish the generation of plasmin, as indicated by a reduction of cleaved chromogenic substrate. This assay was used to analyze the inhibitory capacity of a variety of proteins and peptides, respectively, on the uPA/uPAR interaction: i) uPAR and uPAR-variants expressed in CHO cells, yeast or E. coli, ii) the aminoterminal fragment (**ATF**) of human uPA or yeast recombinant pro-uPA, iii) synthetic peptides derived from the sequence of the uPAR-binding region of uPA, and iv) antibodies directed against uPAR. This assay may be helpful in identifying uPA and uPAR analogues or antagonists which efficiently block uPA/uPAR interaction.

L15 ANSWER 84 OF 129 MEDLINE on STN

96107172. PubMed ID: 8530448. **Urokinase**-type plasminogen activator-induced monocyte adhesion requires a carboxyl-terminal lysine and cAMP-dependent signal transduction. Li C; Liu J N; Gurewich V. (Vascular Research Laboratory, Deaconess Hospital, Harvard Medical School, Boston, Massachusetts 02215, USA.) Journal of biological chemistry, (1995 Dec 22) 270 (51) 30282-5. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB **Urokinase**-type plasminogen activator (u-PA) or its **amino-terminal fragment** (**ATF**) containing the u-PA receptor (u-PAR) binding domain, is known to promote monocyte adhesion. In the present study, U937 monocyte adhesion to a plastic surface was used to investigate the mechanism of its promotion by u-PA and **ATF**. Adhesion was found to be inhibited by cycloheximide or actinomycin D, implicating protein synthesis and gene expression in u-PA-induced monocyte adhesion. Adhesion was prevented by 2'-deoxyadenosine 3'-monophosphate, indicating that a cAMP-dependent pathway of signal transduction was involved. This concept was supported by the complementary finding that u-PA-induced adhesion was greatly promoted by forskolin, cholera toxin, or 8-bromo-cAMP, which by themselves induced little adhesion. Furthermore, similar to many other cAMP-dependent activities, cGMP diminished u-PA-induced adhesion. When u-PA or **ATF** was treated with immobilized carboxypeptidase B, its proadhesive effect was abolished, implicating the involvement of carboxyl-terminal lysine residues (Lys158 on u-PA and Lys135 on **ATF**). Moreover, when a carboxyl-terminal lysine analog was added, the proadhesive effect of carboxypeptidase B-treated u-PA or **ATF** was restored. In conclusion, the present study indicates that u-PA- or **ATF**-induced monocyte adhesion involves cAMP-dependent signal transduction, which is triggered by u-PAR binding. It is also critically dependent on the presence of a carboxyl-terminal lysine.

L15 ANSWER 85 OF 129 MEDLINE on STN

96049541. PubMed ID: 7586807. Increase of a **urokinase** receptor-related low-molecular-weight molecule in colorectal adenocarcinomas. Lau H K; Kim M; Koo J; Chiu B; Murray D. (Division of Hematology and Oncology, St Michael's Hospital, Toronto, Ontario, Canada.) Clinical & experimental metastasis, (1995 Nov) 13 (6) 492-8. Journal code: 8409970. ISSN: 0262-0898. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Proteolytic activity is important for tumor growth and metastasis. Plasminogen and **urokinase**-type plasminogen activator (u-PA) constitute one of the most extensively studied proteolytic systems believed to participate in these processes. u-PA cleaves plasminogen to plasmin, which in turn degrades surrounding extracellular matrix and allows tumor cells to migrate to other areas. The specific receptor for u-PA (u-PAR) has also been implicated as an essential modulator in this pathway. Eleven paired samples of colorectal cancers and normal mucosal tissues from the same patients were removed at surgery. The tissues were homogenized and the supernatants assayed for u-PAR immunoreactivity, u-PAR antigen concentration, u-PAR binding activity and u-PA activity. Immunoblot analysis showed that a major u-PAR species of approximately 55 kDa was present in all tissues. In addition, a protein band of approximately 41 kDa, which crossreacted with anti-u-PAR antibodies, was also found in the tumors. This protein band was either absent, or present in relatively small amounts in the normal colorectal tissues. Cross-linking experiments showed that the approximately 55 kDa band only, and not the approximately 41 kDa band, was able to bind either single chain **urokinase**-type plasminogen activator (scu-PA) or the **amino terminal fragment** of **urokinase** (**ATF**). The tumor samples also exhibited highly elevated u-PA activity and u-PAR antigen relative to the corresponding normal tissues. Elevated u-PA activity appeared to correlate with elevated u-PAR antigen in colorectal cancers, but not in the normal tissues. These increases were also associated with increase of the u-PAR-related, low-molecular-weight protein in the tumor samples. The measurement of u-PAR and the u-PAR-related protein, in addition to u-PA activity, could have diagnostic or prognostic value in this type of cancer.

L15 ANSWER 86 OF 129 MEDLINE on STN

95349959. PubMed ID: 7624151. Heterodimerization of c-Jun with **ATF**-2 and c-Fos is required for positive and negative regulation of the human **urokinase** enhancer. De Cesare D; Vallone D; Caracciolo A; Sassone-Corsi P; Nerlov C; Verde P. (International Institute of Genetics and Biophysics, Naples, Italy.) Oncogene, (1995 Jul 20) 11 (2) 365-76. Journal code: 8711562. ISSN: 0950-9232. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Dimerization plays a pivotal role in modulating the activity of the c-Jun proto-oncogene product. Heterodimerization with activating transcription factor-2 (**ATF**-2) alters the DNA-binding specificity of c-Jun, allowing its targeting to several cAMP responsive element (CRE)-related sequences, which control a subset of AP-1-responsive genes. Here we show that a c-Jun/**ATF**-2 heterodimer binds to the AP-1 site (uPA 5'-TRE) essential for the activity of the human **urokinase** enhancer, conferring on this element several distinctive regulatory properties. The c-Jun/**ATF**-2 heterodimer was identified by binding competition assays, u.v. cross linking, and monospecific antibodies. In vitro binding studies revealed that the uPA 5'-TRE sequence is recognized by the cyclic AMP-unresponsive **ATF**-2 factor, but not by the cyclic AMP-inducible CREB. In addition, in vivo studies suggest that **ATF**-2 can mediate, at the same time, the activation of the c-Jun/**ATF**-2 site and the repression of the canonical collagenase AP-1 site. We report that heterodimerization with c-Fos does not increase the binding of c-Jun to the uPA 5'-TRE, in contrast to the increased binding at a consensus AP-1 site. Our data further suggest that c-Fos can act as a repressor of the c-Jun/**ATF**-2 binding site, revealing an important functional difference, with respect to canonical AP-1 elements.

L15 ANSWER 87 OF 129 MEDLINE on STN

95335838. PubMed ID: 7611439. **Urokinase** receptor in human malignant mesothelioma cells: role in tumor cell mitogenesis and proteolysis. Shetty S; Kumar A; Johnson A; Pueblitz S; Idell S. (Department of Medicine, University of Texas Health Science Center at Tyler 75710, USA.) American journal of physiology, (1995 Jun) 268 (6 Pt 1) L972-82. Journal code: 0370511. ISSN: 0002-9513. Pub. country: United States. Language: English.

AB **Urokinase** (uPA) interacts with its receptor (uPAR) to promote proteolysis and tumor migration, functions of potential importance in the

pathogenesis of malignant mesothelioma. Immunohistochemistry of human malignant mesothelioma tissue and mesothelioma cells (MS-1) showed that mesothelioma cells express uPAR. We isolated uPAR from MS-1 cells by metabolic labeling and showed that it could be induced by phorbol myristate acetate (PMA), lipopolysaccharide (LPS), a transforming growth factor-beta (TGF-beta) or tumor necrosis factor-alpha (TNF-alpha). Experiments with MS-1 cells showed that uPA binding was saturable, specific, and reversible with a mean dissociation constant (Kd) of 5.4 +/- 1.1 nM. Binding was inhibited by a blocking antibody to uPAR and by the uPA **amino-terminal fragment** (**ATF**), but not by low molecular weight uPA. uPAR expression was regulated transcriptionally and translationally; antisense oligonucleotides blocked expression of uPAR protein. Plasminogen activator inhibitor-1 (PAI-1) inhibited PA activity of preformed uPA/uPAR complexes and increased cycling of the receptor from the cell surface. Stimulation of subconfluent MS-1 cells by high molecular weight or recombinant uPA, but not **ATF** or low molecular weight fragment, caused concentration-dependent incorporation of [3H]thymidine. These data indicate a novel mechanism by which malignant mesothelioma cells localize pericellular proteolysis and concurrently regulate tumor cell proliferation.

L15 ANSWER 88 OF 129 MEDLINE on STN

95329570. PubMed ID: 7605874. Studies of possible mechanisms for the effect of **urokinase** therapy in small cell carcinoma of the lung. Meehan K R; Zacharski L R; Maurer L H; Howell A L; Memoli V A; Rousseau S M; Hunt J A; Henkin J. (Department of Veterans Affairs Medical & Regional Office Center, White River Junction, Vermont 05009, USA.) Blood coagulation & fibrinolysis : an international journal in haemostasis and thrombosis, (1995 Apr) 6 (2) 105-12. Journal code: 9102551. ISSN: 0957-5235. Pub. country: ENGLAND: United Kingdom. Language: English.

AB **Urokinase**-type plasminogen activator has been administered by other investigators to patients with small cell carcinoma of the lung (SCCL) in an attempt to induce lysis of fibrin that is known to exist in the connective tissue stroma of this tumour type and that may support tumour growth. To study the fate of infused **urokinase** in this disease, a biopsy of a scalp metastasis was obtained from a patient with SCCL (entered on a phase I clinical trial of **urokinase** plus combination chemotherapy) immediately following **urokinase** infusion during the fourth course of therapy a time when this tumour mass had decreased to approximately 25% of its original size. Immunohistochemical procedures revealed abundant stromal fibrin in accord with previous observations from this laboratory. By contrast, **urokinase**, that is not a feature of small cell tumour cells, was present on the tumour cells in this specimen. **Urokinase** infusion was associated with a rapid increase in the amount of this enzyme associated with isolated peripheral blood monocytes. These results are consistent with uptake of infused **urokinase** onto monocytes and possibly tumour cells. It is postulated that substantial tumour fibrinolysis may not accompany such therapy and that **urokinase**, or its **amino terminal fragment** that bears the growth factor domain of this molecule, may bind to and alter the growth of the tumour cells.

L15 ANSWER 89 OF 129 MEDLINE on STN

95229658. PubMed ID: 7713945. Inhibitory effect of a conjugate between human **urokinase** and urinary trypsin inhibitor on tumor cell invasion in vitro. Kobayashi H; Gotoh J; Hirashima Y; Fujie M; Sugino D; Terao T. (Department of Obstetrics and Gynecology, Hamamatsu University School of Medicine, Shizuoka, Japan.) Journal of biological chemistry, (1995 Apr 7) 270 (14) 8361-6. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Proteolytic enzymes such as **urokinase**-type plasminogen activator (uPA), plasmin, and collagenase mediate proteolysis by a variety of tumor cells. uPA secreted by tumor cells can be bound to a cell surface receptor via a growth factor-like domain within the **amino-terminal fragment** (**ATF**) of the uPA molecule with high affinity. Urinary trypsin inhibitor (UTI) efficiently inhibits the soluble and the tumor cell-surface receptor-bound plasmin and subsequently reduces tumor cell invasion and the formation of

metastasis. The anti-invasive effect is dependent on the anti-plasmin activity of the UTI molecule, domain II in particular. We synthesized a conjugate between **ATF** of human uPA and a native UTI molecule or domain II of UTI (HI-8). The effect of the conjugates (**ATF**.UTI or **ATF**.HI-8) on tumor cell invasion in vitro was investigated. **ATF**.UTI and **ATF**.HI-8 bound to U937 cells in a rapid, saturable, dose-dependent, and reversible manner. A large part of receptor-bound **ATF**-UTI and **ATF**.HI-8 remains on the cell surface for at least 5 h at 37 degrees C. Inhibition of tumor cell-surface receptor-bound plasmin by **ATF**.UTI and **ATF**.HI-8 was markedly enhanced when compared with tumor cells treated either with **ATF**, UTI, or HI-8. Results of a cell invasion assay showed that **ATF**.UTI and **ATF**.HI-8 is very effective at targeting HI-8 specifically to uPA receptor-expressing tumor cells, whereas tumor cells devoid of uPA receptor may be less affected by the conjugates. Our results indicate that cell surface uPA and plasmin activity is essential to the invasive process and that the conjugates exhibit plasmin inhibition to the close environment of the cell surface and subsequently inhibit the tumor cell invasion through Matrigel in an in vitro invasion assay.

L15 ANSWER 90 OF 129 MEDLINE on STN

95213999. PubMed ID: 7699636. Control of the chondrocyte fibrinolytic balance by the drug piroxicam: relevance to the osteoarthritic process. Fibbi G; Serni U; Matucci A; Mannoni A; Pucci M; Anichini E; Del Rosso M. (Istituto di Patologia Generale, Universita di Firenze, Italy.) Journal of rheumatology, (1994 Dec) 21 (12) 2322-8. Journal code: 7501984. ISSN: 0315-162X. Pub. country: Canada. Language: English.

AB OBJECTIVE. Since the plasminogen activator [PA/plasminogen activator inhibitor (PAI) system is believed to be involved in a breakdown of articular cartilage in osteoarthritis (OA), we studied the modulation of single components of the fibrinolytic system (**urokinase**-type plasminogen activator, u-PA; plasminogen activator inhibitor-1, PAI-1; the surface receptor for u-PA, u-PAR) in human chondrocytes in the presence of piroxicam. METHODS. The drug was added to the chondrocyte culture medium either directly or by supplementing chondrocyte cultures with synovial fluid (SF) from patients with OA treated with piroxicam. We have shown u-PAR M(r) 55000 Da on human chondrocytes in suspension culture by cross linking chondrocyte lysates with 125I-labelled **amino-terminal fragment** (**ATF**) of human u-PA, which frames the sequence that specifically interacts with u-PAR. RESULTS. Such receptors decrease upon incubation of chondrocytes with piroxicam or with SF from patients treated with piroxicam. The culture medium of treated chondrocytes showed decreased fibrinolytic activity when compared with untreated controls, while PAI activity was increased in both SF chondrocyte culture medium following piroxicam treatment. At the same time, internalization of u-PA/u-PAR complexes increased after incubation of chondrocytes with piroxicam or PAI-1 rich SF. CONCLUSION. Our results indicate that the drug induces the surface clearance u-PAR by internalization of u-PA/PAI-/u-PAR complexes. Thus piroxicam reduces both the soluble fibrinolytic activity of human chondrocytes (increase of PAI activity and decrease of released u-PA) and the cell associated u-PA activity (clearance of u-PAR by internalization). The drug dependent changes in the fibrinolytic system suggest that piroxicam may be useful in preventing or limiting perilacunar cartilage damage in OA.

L15 ANSWER 91 OF 129 MEDLINE on STN

95143384. PubMed ID: 7841301. Soluble **urokinase** receptor from fibrosarcoma HT-1080 cells. Lau H K; Kim M. (Division of Hematology, St Michael's Hospital, Toronto, Ontario, Canada.) Blood coagulation & fibrinolysis : an international journal in haemostasis and thrombosis, (1994 Aug) 5 (4) 473-8. Journal code: 9102551. ISSN: 0957-5235. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A soluble form of **urokinase**-binding protein has been isolated from the human fibrosarcoma cell line HT-1080 and cell lines derived from it. Conditioned media of these cells were collected after overnight incubation under serum-free conditions, and were concentrated and passed through a column of Sepharose 4B to which high-molecular-weight **urokinase** had been

attached. After thorough washing, a polypeptide could be eluted from the column with 1 M acetic acid. This material appeared to be a single band of approximately 60 kDa on SDS polyacrylamide gel. It cross-reacted with commercial antibodies made against **urokinase** receptor, and could be chemically cross-linked to the **amino terminal fragment** of **urokinase**. This material was similar to the **urokinase** receptor that was cleaved from HT-1080 cells by means of phosphatidylinositol-specific phospholipase C.

L15 ANSWER 92 OF 129 MEDLINE on STN
95101635. PubMed ID: 7803405. Backbone dynamics of a two-domain protein: 15N relaxation studies of the **amino-terminal fragment** of **urokinase**-type plasminogen activator. Hansen A P; Petros A M; Meadows R P; Fesik S W. (Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, Ilinois 60064.) Biochemistry, (1994 Dec 27) 33 (51) 15418-24. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.
AB The **amino-terminal fragment** (**ATF**) of **urokinase**-type plasminogen activator (u-PA) is a two-domain protein which consists of a kringle and a growth factor domain (GFD). The dynamics of uniformly 15N-labeled **ATF** was examined by measuring the longitudinal (T1) and transverse (T2) 15N relaxation times and heteronuclear NOEs. The data were interpreted in terms of the model-independent spectral density function. The GFD was found to exhibit a high degree of anisotropy, whereas the kringle domain of **ATF** undergoes isotropic reorientation. This difference in anisotropy is best explained by the two domains moving independently such as differently shaped beads on a string. With the exception of the N- and C-terminal regions of the protein, the most flexible region of **ATF** was the seven-residue omega loop (N22-I28) of the GFD which has been implicated in the binding of u-PA to its receptor. The amides of the linker region between the domains displayed high values of the order parameter, indicating restricted motion on the picosecond time scale. This is in contrast to the flexible linker of calmodulin [Barbato et al. (1992) Biochemistry 31, 5269-5278], which displayed low values of S2 and unrestricted motion in the linker region.

L15 ANSWER 93 OF 129 MEDLINE on STN
95080428. PubMed ID: 7988721. Blockage of the **urokinase** receptor on the cell surface: construction and characterization of a hybrid protein consisting of the N-terminal fragment of human **urokinase** and human albumin. Lu H; Yeh P; Guitton J D; Mabilat C; Desanlis F; Maury I; Legrand Y; Soria J; Soria C. (Unite INSERM 353, Hopital St. Louis, Paris, France.) FEBS letters, (1994 Dec 12) 356 (1) 56-9. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.
AB Receptor-bound **urokinase** is likely to be a crucial determinant in both tumor invasion and angiogenesis. We report here that a yeast-derived genetic conjugate between human serum albumin and the 1-135 N-terminal residues of **urokinase** (u-PA) competitively inhibits the binding of exogenous and endogenous u-PA to its cell-anchored receptor (u-PAR). This hybrid molecule (**ATF**-HSA) also inhibits in vitro pro-**urokinase**-dependent plasminogen activation in the presence of u-PAR bearing cells. These effects are probably responsible for the observed in vitro inhibition of tumor cell invasion in a reconstituted basement membrane extract (Matrigel).

L15 ANSWER 94 OF 129 MEDLINE on STN
95019438. PubMed ID: 7933839. Receptor binding and degradation of **urokinase**-type plasminogen activator by human mesangial cells. Nguyen G; Li X M; Peraldi M N; Zacharias U; Hagege J; Rondeau E; Sraer J D. (INSERM U 64, Hopital Tenon, Paris, France.) Kidney international, (1994 Jul) 46 (1) 208-15. Journal code: 0323470. ISSN: 0085-2538. Pub. country: United States. Language: English.
AB The binding of [125I] labeled **urokinase**-type plasminogen activator (u-PA) was studied on human mesangial cells (MC) in culture. The binding of active [125I]u-PA at 37 degrees C reached a plateau after 30 minutes of incubation and remained stable for at least four hours. When the

supernatant was analyzed with trichloracetic acid (TCA), TCA soluble radioactive material could be detected after a lag phase of 30 minutes, and then increased linearly for four hours. Analysis by electrophoresis on SDS PAGE and autoradiography of the cell associated radioactivity and of the intracellular content showed that active u-PA and u-PA complexed to plasminogen activator inhibitor type-1 (PAI-1) were bound to the cell surface, but only u-PA/PAI-1 complexes were internalized and degraded. Therefore, the Kd and the number of binding sites were determined by competitive inhibition curves at 4 degrees C using diisopropyl-fluorophosphate (DFP) u-PA. Scatchard plots showed a Kd = 400 +/- 30 pM, and Bmax = 240,000 +/- 25,000 sites/cell. Excess of the **amino terminal fragment** of u-PA (**ATF**) completely blocked the specific binding of [125I]u-PA, confirming that the binding of u-PA was independent of the presence of the active site and/or of the formation of complexes with PAI-1. 3H thymidine incorporation by mesangial cells after stimulation with 100 nM active u-PA showed that u-PA had a moderate but significant mitogenic effect, in contrast to inactive u-PA and **ATF**. However, this mitogenic effect was not accompanied by a proliferative effect.(ABSTRACT TRUNCATED AT 250 WORDS)

L15 ANSWER 95 OF 129 MEDLINE on STN

94326845. PubMed ID: 8050501. Production of second messengers following chemotactic and mitogenic **urokinase**-receptor interaction in human fibroblasts and mouse fibroblasts transfected with human **urokinase** receptor. Anichini E; Fibbi G; Pucci M; Caldini R; Chevanne M; Del Rosso M. (Institute of General Pathology, Florence University, Italy.) Experimental cell research, (1994 Aug) 213 (2) 438-48. Journal code: 0373226. ISSN: 0014-4827. Pub. country: United States. Language: English.

AB We studied **urokinase**-type plasminogen activator (u-PA)-dependent chemotaxis and DNA synthesis in both human fibroblasts and LB6 mouse fibroblasts transfected with human u-PA receptor (u-PAR) gene (LB6 clone 19). Both cell lines have receptors for the **amino-terminal fragment** of u-PA (u-PA-**ATF**). We observed that u-PA and u-PA-**ATF** stimulated chemotactic migration of both LB6 clone 19 cells and human fibroblasts, which could be impaired by down-regulation of protein kinase C (PKC) with phorbol myristate acetate (PMA). While LB6 clone 19 cells were unable to undergo mitosis following exposure to either u-PA or u-PA-**ATF**, human fibroblasts were stimulated to mitosis by exogenous addition of native u-PA, and u-PA-**ATF** was ineffective. The mitogenic activity of u-PA on human fibroblasts could also be impaired by down-regulation of PKC with PMA. We studied second messenger formation following u-PAR stimulation. Neither inositol lipid metabolism nor intracellular Ca2+ content were affected, while an increase of diacylglycerol (DAG) generation was observed. Such DAG formation was related to de novo synthesis from glucose and was dependent on ligand-receptor interaction. Both u-PA-**ATF** and the native u-PA molecule were able to stimulate DAG formation, u-PA being from three to fourfold more efficient than **ATF**. These data suggest that u-PAR stimulation per se is sufficient to trigger DAG formation. The native molecule confers on the cell an additional stimulus, possibly related with the activation of a u-PA-catalytic site-dependent substrate. Such stimulation allows the cell to reach the DAG threshold level required to trigger DNA synthesis.

L15 ANSWER 96 OF 129 MEDLINE on STN

94318644. PubMed ID: 8043585. Ligand interaction between **urokinase**-type plasminogen activator and its receptor probed with 8-anilino-1-naphthalenesulfonate. Evidence for a hydrophobic binding site exposed only on the intact receptor. Ploug M; Ellis V; Dano K. (Finsen Laboratory, Rigshospitalet, Copenhagen O, Denmark.) Biochemistry, (1994 Aug 2) 33 (30) 8991-7. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB The cellular receptor for **urokinase**-type plasminogen activator (uPAR) is a glycolipid-anchored membrane protein thought to play a primary role in the generation of pericellular proteolytic activity, and to be involved in cancer cell invasion and metastasis. This protein is composed of three homologous domains, the NH2-terminal of which is involved in the

high affinity binding (Kd approximately 0.1-1.0 nM) to the epidermal growth factor-like module of **urokinase**-type plasminogen activator (uPA). Here we report that intact uPAR binds the low molecular weight fluorophore 8-anilino-1-naphthalenesulfonate (ANS) to form a 1:1 stoichiometric complex and that the resulting enhancement of the ANS fluorescence probes the functional state of uPAR as judged by several independent criteria. First, the uPAR-mediated increase in ANS fluorescence can be titrated by uPA as well as by its receptor binding derivatives (the **amino-terminal fragment** and the growth factor-like module). Second, an anti-uPAR monoclonal antibody, capable of preventing uPA binding, can also titrate the uPAR-dependent ANS fluorescence whereas other antibodies not interfering with uPA binding are unable to exert this effect. Third, the dissociation profile of uPA-uPAR complexes as a function of increasing concentrations of guanidine hydrochloride closely parallels the loss of the ANS binding site in uPAR. Finally, liberation of the NH2-terminal domain from uPAR by limited chymotrypsin cleavage after Tyr87 leads to a loss of both enhanced ANS fluorescence and high-affinity uPA binding.(ABSTRACT TRUNCATED AT 250 WORDS)

L15 ANSWER 97 OF 129 MEDLINE on STN

94299497. PubMed ID: 8027043. Protease nexin-1-**urokinase** complexes are internalized and degraded through a mechanism that requires both **urokinase** receptor and alpha 2-macroglobulin receptor. Conese M; Olson D; Blasi F. (Department of Biological and Technological Research, San Raffaele Research Institute, Milano, Italy.) Journal of biological chemistry, (1994 Jul 8) 269 (27) 17886-92. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB After binding to its receptor (uPAR), active cell-surface **urokinase** (uPA) is not internalized while the complex formed by uPA with plasminogen activator inhibitor type 1 (PAI-1) is internalized and degraded. Internalization and degradation require binding to uPAR and subsequently an interaction with the alpha 2-macroglobulin receptor (alpha 2-MR). To analyze the generality of this mechanism, we studied the internalization of uPA by recombinant protease nexin-1 (rPN-1), an inhibitor of thrombin, uPA, and plasmin. 125I-uPA.rPN-1 complexes bound specifically to uPAR; internalization occurred efficiently, and its time course was essentially the same as for uPA.PAI-1. Internalization required binding to uPAR since it could be blocked by the anti-uPAR monoclonal antibodies, by the uPAR antagonist **amino-terminal fragment** of uPA, and by the removal of uPAR by the treatment of cells with phosphatidylinositol-specific phospholipase C. As for uPA.PAI-1, the internalization of uPA.rPN-1 also required alpha 2-MR, since it could be inhibited by the 39-kDa alpha 2-macroglobulin receptor/low density lipoprotein receptor-associated protein, a ligand for the alpha 2-MR. Finally, we show by ligand blot analysis that the uPA.rPN-1 complex, like uPA.PAI-1 but unlike free uPA, bound specifically to both uPAR and alpha 2-MR.

L15 ANSWER 98 OF 129 MEDLINE on STN

94291776. PubMed ID: 8020601. **Urokinase**-type and tissue-type plasminogen activators as growth factors of human fibroblasts. De Petro G; Copeta A; Barlati S. (Department of Biomedical Sciences and Biotechnologies, University of Brescia, Italy.) Experimental cell research, (1994 Jul) 213 (1) 286-94. Journal code: 0373226. ISSN: 0014-4827. Pub. country: United States. Language: English.

AB In this study we have verified the mitogenic effect of **urokinase**-type (u-PA) and tissue-type plasminogen activators (t-PA) on human normal fibroblasts. We report that both PAs can induce DNA replication and cell division in serum-deprived cultured human skin fibroblasts. The activity of u-PA and t-PA is, respectively, three- and twofold more potent than that exerted by epidermal growth factor (EGF) with an activity slightly lower (50-60%) than that induced by basic fibroblast growth factor (bFGF). The u-PA and t-PA, but not plasmin, induced DNA synthesis, which could be neutralized by anti-u-PA and anti-t-PA antibodies, respectively, but was insensitive to aprotinin treatment. The addition of anti-u-PA-receptor (u-PAR) monoclonal antibodies to the assays selectively suppressed the mitogenic effect exerted by u-PA, but not that of t-PA, and the

amino terminal fragment of u-PA, containing the EGF-like domain and the kringle module, did not elicit any mitogenic activity. Anti-bFGF antibodies completely suppressed the mitogenic activity of bFGF, but did not have any effect on that of u-PA and t-PA; the activity of both PAs was inhibited by anti-fibronectin IgG concentrations ineffective on bFGF. These results indicate that PAs may be considered growth factors of human fibroblasts.

L15 ANSWER 99 OF 129 MEDLINE on STN

94243832. PubMed ID: 8187097. Binding of **urokinase** to its receptor promotes migration and invasion of human melanoma cells in vitro. Stahl A; Mueller B M. (Department of Immunology, Scripps Research Institute, La Jolla, California 92037.) Cancer research, (1994 Jun 1) 54 (11) 3066-71. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB Previously, we reported that **urokinase**-type plasminogen activator (uPA) plays a pivotal role in extracellular matrix dissolution by malignant melanoma cells. Here, we demonstrate that a highly metastatic melanoma cell line (M24met) that secretes uPA expresses high levels of the uPA receptor (uPAR), 2.4 x 10(6) binding sites/cell with a KD of 5.67 x 10(-10) M. The receptor was identified as a 55,000-60,000 kDa cell surface protein. Although M24met cells secrete uPA, they are unable to efficiently utilize this enzyme for invasion, unless it is bound to its receptor. This contention is based on the finding that an antibody against uPAR (monoclonal antibody 3936) inhibited invasion of M24met cells through a reconstituted basement membrane (Matrigel) up to 33%, while a reduction of uPA catalytic activity by its plasminogen activator inhibitor-2 resulted in 46% inhibition of invasion. Furthermore, uPAR is involved in signal transduction events in M24met cells, since both uPA and its **amino-terminal fragment** stimulated the migration of melanoma cells toward Matrigel, resulting in maximal increases of 32 and 72%, respectively. Our results indicate that both uPA and uPAR are involved in melanoma metastasis and that uPAR contributes to at least two important steps in this process, matrix dissolution and migration.

L15 ANSWER 100 OF 129 MEDLINE on STN

94213863. PubMed ID: 8161544. Solution structure of the **amino-terminal fragment** of **urokinase**-type plasminogen activator. Hansen A P; Petros A M; Meadows R P; Nettesheim D G; Mazar A P; Olejniczak E T; Xu R X; Pederson T M; Henkin J; Fesik S W. (Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, Illinois 60064.) Biochemistry, (1994 Apr 26) 33 (16) 4847-64. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB The **amino-terminal fragment** (**ATF**) of **urokinase**-type plasminogen activator is a two domain protein which consists of a growth factor and a kringle domain. The 1H, 13C, and 15N chemical shifts of this protein have been assigned using heteronuclear two- and three-dimensional NMR experiments on selective and uniformly 15N- and 15N/13C-labeled protein isolated from mammalian cells that overexpress the protein. The chemical shift assignments were used to interpret the NOE data which resulted in a total of 1299 NOE restraints. The NOE restraints were used along with 27 phi angle restraints and 21 hydrogen-bonding restraints to produce 15 low energy structures. The individual domains in the structures are highly converged, but the two domains are structurally independent. The root mean square deviations (rmsd) between residues 11-46 in the growth factor domain and the mean atomic coordinates were 0.99 +/- 0.2 for backbone heavy atoms and 1.65 +/- 0.2 for all non-hydrogen atoms. For residues 55-130 in the kringle domain, the rmsd was 0.84 +/- 0.2 for backbone heavy atoms and 1.42 +/- 0.2 for all non-hydrogen atoms. The overall structures of the individual domains are very similar to the structures of homologous proteins. However, important structural differences between the growth factor and other homologous proteins were observed in the region which has been implicated in binding the **urokinase** receptor which may explain, in part, why other growth factors show no appreciable affinity for the **urokinase** receptor.

L15 ANSWER 101 OF 129 MEDLINE on STN
94171881. PubMed ID: 8126064. Requirement for receptor-bound **urokinase** in plasmin-dependent cellular conversion of latent TGF-beta to TGF-beta. Odekon L E; Blasi F; Rifkin D B. (Department of Cell Biology, New York University Medical Center, New York.) Journal of cellular physiology, (1994 Mar) 158 (3) 398-407. Journal code: 0050222. ISSN: 0021-9541. Pub. country: United States. Language: English.

AB The role of receptor-bound **urokinase**-type plasminogen activator (uPA) in cellular activation of latent transforming growth factor-beta (LTGF-beta) was investigated in a model system of mouse LB6 cells transfected with either a human uPA receptor cDNA (LhuPAR+), a human prouPA cDNA (LhuPA), or a control neomycin-resistance cDNA (Lneo). When LhuPAR+ cells were co-cultured with LhuPA cells, the plasmin-dependent fibrinolytic activity generated was more than that observed in either homotypic cultures with fivefold greater number of LhuPA cells or co-cultures containing LhuPA and Lneo cells instead of the LhuPAR+ cells. The preferential activation of TGF-beta by co-cultures with the greatest plasmin-generation potential, LhuPAR+ and LhuPA cells, was confirmed by three independent bioassays. In the first assay, a 48% decrease in PA activity, a measure of active TGF-beta production, was observed with BAE cells treated with conditioned medium (CM) from co-cultures of LhuPA and LhuPAR+ cells. Inclusion of neutralizing antibodies to TGF-beta abrogated the inhibitory effect of CM on PA activity demonstrating that the inhibitory molecule was TGF-beta. Addition of the **amino terminal fragment** of uPA (**ATF**) or omission of plasminogen from co-cultures blocked both the fibrinolytic activity and the generation of TGF-beta activity in the CM. In the second assay, CM from co-cultures of LhuPA and LhuPAR+ cells inhibited the migration of BAE cells in a wound assay. Controls with anti-TGF-beta IgG indicated that the inhibition was due to TGF-beta. In the third assay, proliferation of mink lung epithelial cells was inhibited by CM generated by co-cultures of LhuPA and LhuPAR+ cells as compared to CM from the same cells cultured in the absence of plasminogen or to CM from a co-culture of LhuPA with LhuPAR- cells. Excess mannose-6-phosphate (M6P) blocked the generation of TGF-beta as assayed by both the BAE migration and PA assays, presumably because it interfered with cell-surface localization of LTGF-beta. Additionally, small numbers of LhuPA and LhuPAR+ cells co-cultured with BAE cells inhibited the BAE cell PA activity via the paracrine action of TGF-beta. These results support the conclusion that plasmin-dependent activation LTGF-beta by LB6 cells is promoted by the surface localization of uPA by its receptor.

L15 ANSWER 102 OF 129 MEDLINE on STN
94012773. PubMed ID: 7691818. Low density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor mediates cellular uptake of pro-**urokinase**. Kounnas M Z; Henkin J; Argraves W S; Strickland D K. (Biochemistry Laboratory, American Red Cross, Rockville, Maryland 20855.) Journal of biological chemistry, (1993 Oct 15) 268 (29) 21862-7. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The low density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor (LRP) is a large cell surface receptor consisting of a 515-kDa heavy chain and an 85-kDa light chain proteolytically derived from a 600-kDa precursor. Previous work has shown that LRP is responsible for mediating the internalization of urinary-type plasminogen activator (uPA) complexed to plasminogen activator inhibitor type I (PAI-1) (Nykjaer et al., 1992; Herz et al., 1992). The current study indicates that pro-**urokinase** (pro-uPA) and two chain **urokinase** (tc-uPA) bind directly to purified LRP, and that LRP mediates their internalization and degradation in Hep G2 cells. In vitro binding assays demonstrated that pro-uPA and tc-uPA bind to purified LRP with affinities (Kd = 45 and 60 nM, respectively) that are approximately 15 to 20-fold weaker than the affinity of uPA.PAI-1 complex for LRP (Kd = 3 nM). Competitive binding experiments revealed that pro-uPA and tc-uPA completely inhibit binding of uPA.PAI-1 complexes to purified LRP. The binding of 125I-pro-uPA to LRP is blocked by the 39-kDa receptor-associated protein, but not by an **amino-terminal fragment** of uPA, which is known to block binding of

uPA to the **urokinase** receptor. 125I-PIO-uPA can be internalized and degraded by Hep G2 cells independent of PAI-1. Both the internalization and degradation are completely blocked by receptor-associated protein or affinity-purified LRP antibodies, indicating that LRP is mediating this process. These processes are also blocked by the **amino-terminal fragment**, which suggests that the favored pathway for uPA metabolism is initial binding to the **urokinase** receptor, followed by ligand transfer to LRP, then internalization leading to degradation.

L15 ANSWER 103 OF 129 MEDLINE on STN
93272971. PubMed ID: 8388810. Processing of complex between **urokinase** and its type-2 inhibitor on the cell surface. A possible regulatory mechanism of **urokinase** activity. Ragno P; Montuori N; Vassalli J D; Rossi G. (Centro di Endocrinologia ed Oncologia Sperimentale (CEOS), Consiglio Nazionale delle Ricerche, Naples, Italy.) FEBS letters, (1993 Jun 1) 323 (3) 279-84. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.
AB Complexes between the **urokinase**-type plasminogen activator (uPA) and its type-2 inhibitor (PAI-2) are bound by a cell-surface receptor for uPA and rapidly cleaved into two fragments of 70 and 22 kDa. The 70-kDa fragment contains the active site of uPA and PAI-2, while the 22-kDa species was identified as the **amino terminal fragment** of uPA, that binds specifically to the receptor. When the experiment is performed at 4 degrees C, both fragments remain bound to the cell surface and can be eluted by acid treatment. We therefore postulate that after the binding of the uPA-PAI-2 complex, a new binding site for the 70-kDa species becomes available. This additional binding favours the cleavage of the complex into the 70-and 22-kDa fragments; the 70-kDa species is endocytosed or released, while the 22-kDa fragment remains on the cell surface to prevent the binding of intact uPA.

L15 ANSWER 104 OF 129 MEDLINE on STN
93261817. PubMed ID: 8388098. Purification and cDNA cloning of a transcription factor which functionally cooperates within a cAMP regulatory unit in the porcine uPA gene. Menoud P A; Matthies R; Hofsteenge J; Nagamine Y. (Friedrich Miescher-Institut, Basel, Switzerland.) Nucleic acids research, (1993 Apr 25) 21 (8) 1845-52. Journal code: 0411011. ISSN: 0305-1048. Pub. country: ENGLAND: United Kingdom. Language: English.
AB One of cAMP-regulatory sites in the porcine **urokinase**-type plasminogen activator (uPA) gene resides 3.4 kb upstream of the transcription initiation site and is composed of three protein binding domains, FPA, FPB and FPC. Whereas FPA and FPB contain a CRE-like sequence, the FPC sequence is not related to any known protein recognition sequences, yet all three domains are required to mediate cAMP action on a heterologous promoter. To study the functional cooperation among these three domains we purified and cloned a FPC-binding protein (FPCB) from porcine kidney derived LLC-PK1 cells. Sequence comparisons showed that FPCB is homologous to mouse LFB3 and rat vHNF1. LFB3/vHNF1 is related to a liver specific transcription factor HNF1, it recognizes the same sequence as HNF1 and is highly expressed in kidney cells. FPCB and HNF1 recognition sequences are dissimilar, nevertheless both sequences are recognized by in vitro-translated LFB3 and FPCB, indicating that binding to the two different sequences is an intrinsic character of FPCB/LFB3/vHNF1. In HeLa cells, this cAMP-responsive site was inactive whether FPCB was overexpressed or not, suggesting a requirement for an additional cell-specific factor. These results may suggest a mechanism by which hormonal control is integrated into cell-specific gene regulation.

L15 ANSWER 105 OF 129 MEDLINE on STN
93176695. PubMed ID: 8382511. Saturation of tumour cell surface receptors for **urokinase**-type plasminogen activator by **amino-terminal fragment** and subsequent effect on reconstituted basement membranes invasion. Kobayashi H; Ohi H; Shinohara H; Sugimura M; Fujii T; Terao T; Schmitt M; Goretzki L; Chucholowski N; Janicke F; +. (Department of Obstetrics and Gynecology, Hamamatsu University School of Medicine,

Shizuoka, Japan.) British journal of cancer, (1993 Mar) 67 (3) 537-44. Journal code: 0370635. ISSN: 0007-0920. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Single-chain **urokinase**-type plasminogen activator (pro-uPA) is bound to a specific surface receptor on ovarian cancer HOC-I cells that is incompletely saturated. Saturation of uncovered receptors by uPA polypeptides with intact **amino-terminal fragment** (**ATF**) derived from pro-uPA by limited proteolysis (human leucocyte elastase [HLE] or V8 protease) has been studied. HOC-I cells preferentially invaded reconstituted basement membranes in a time- and plasminogen-dependent manner. This process was inhibitable by preincubation with uPA polypeptides in the medium at levels which suggested that complete saturation of cell surface uPA receptors occurred. This result indicates that occupation of uPA receptors by enzymatically inactive uPA fragments or prevention of rebinding of pro-uPA synthesised by tumour cells to the receptors specifically reduces the invasion of the tumour cells through basement membranes in vitro.

L15 ANSWER 106 OF 129 MEDLINE on STN

93151813. PubMed ID: 8381273. **Urokinase-urokinase** receptor interaction: non-mitogenic signal transduction in human epidermal cells. Del Rosso M; Anichini E; Pedersen N; Blasi F; Fibbi G; Pucci M; Ruggiero M. (Institute of General Pathology, Firenze, Italy.) Biochemical and biophysical research communications, (1993 Jan 29) 190 (2) 347-52. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB We studied non-mitogenic signal transduction in a human cell line of epidermal origin which is induced to chemotaxis following stimulation with human **urokinase**-type plasminogen activator (u-PA) or with the **amino-terminal fragment** (**ATF**) of u-PA A chain, which specifically interacts with the cellular receptor. U-PA and **ATF** stimulated the formation of diacylglycerol (DAG) independently of inositol lipid and phosphatidylcholine turnover, but concomitantly with de novo synthesis from glucose, thus resembling the DAG neosynthesis activated by insulin. DAG was measured in normal epidermal cells and in cells transfected with the human u-PA receptor (u-PAR) gene and stimulated with u-PA or **ATF**. Transfected clones showed an increase of cell motility under an **ATF** gradient in vitro as well as an increase of DAG production. These findings identify a novel mechanism of second messenger formation that conveys chemotactic signals upon stimulation of the u-PAR.

L15 ANSWER 107 OF 129 MEDLINE on STN

93093104. PubMed ID: 1333982. Selective localization of receptors for **urokinase amino-terminal fragment** at substratum contact sites of an in vitro-established line of human epidermal cells. Del Rosso M; Pedersen N; Fibbi G; Pucci M; Dini G; Anichini E; Blasi F. (Istituto di Patologia Generale, Universita di Firenze, Italy.) Experimental cell research, (1992 Dec) 203 (2) 427-34. Journal code: 0373226. ISSN: 0014-4827. Pub. country: United States. Language: English.

AB We have shown the presence of surface receptors for the **amino-terminal fragment** (**ATF**) of human **urokinase**-type plasminogen activator (u-PA) on an in vitro-established cell line of human epidermal origin by both radio-binding assays with human 125I-u-PA-**ATF** and transmission electron microscopy of a gold-u-PA complex. On the basis of cross-linking experiments with 125I-u-PA-**ATF** and subsequent autoradiography of the gels we have observed that such receptors are not spontaneously released into the culture medium. The treatment with phosphatidylinositol-specific phospholipase C induces the release of the receptor, which behaves as a glycosyl phosphatidyl inositol(GPI)-anchored protein. Phase-partitioning experiments on cell lysates have shown that the receptor partitions into the detergent phase. By detaching cell monolayers with the chelating agent EDTA we have prepared the cell-substratum contact sites of these cells, which represent only the 3.5% of the surface membrane of monolayered cells. Such plasma membrane remnants are highly selected since they contain about 43% of total u-PA-**ATF** binding sites. Such binding sites show the same biochemical and morphological characteristics

of u-PA **ATF** receptors observed in the monolayered cells, thus indicating that u-PA is selectively concentrated at the level of cell-substratum contacts. This is likely to enable directional proteolysis for cell migration and invasion.

L15 ANSWER 108 OF 129 MEDLINE on STN

92378989. PubMed ID: 1510944. Heparin binding to the **urokinase** kringle domain. Stephens R W; Bokman A M; Myohanen H T; Reisberg T; Tapiovaara H; Pedersen N; Grondahl-Hansen J; Llinas M; Vaheri A. (Department of Virology, University of Helsinki, Finland.) Biochemistry, (1992 Aug 25) 31 (33) 7572-9. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB The binding of **urokinase** to immobilized heparin and dextran sulfate was studied using activity assays of the bound **urokinase**. The markedly higher binding observed with high M(r) **urokinase** compared to low M(r) **urokinase** indicated a role for the **amino-terminal fragment** (**ATF**). This was confirmed by the use of inactive truncated **urokinase** and monoclonal antibodies specific for the **ATF** in competition assays of **urokinase** binding. Antibody competition assays suggested a site in the kringle domain, and a synthetic decapeptide Arg-52-Trp-62 from the kringle sequence (kringle numbering convention) was competitive in assays of **urokinase** binding to dextran sulfate and heparin. Heparin binding to the **urokinase** kringle was unambiguously demonstrated via 1H NMR spectroscopy at 500 MHz. Effective equilibrium association constants (K(a)*) were determined for the interaction of isolated kringle fragment and low M(r) heparin at pH 7.2. The binding was strong in salt-free 2H2O (K(a)* approximately 57 mM-1) and remained significant in 0.15 M NaCl (K(a)* approximately 12 mM-1), supporting a potential physiological role for the interaction. This is the first demonstration of a function for the kringle domain of **urokinase**, and it suggests that while the classical kringle structure has specificity for lysine binding, there may also exist a class of kringles with affinity for polyanion binding.

L15 ANSWER 109 OF 129 MEDLINE on STN

92362631. PubMed ID: 1499567. **Urokinase** binding to laminin-nidogen. Structural requirements and interactions with heparin. Stephens R W; Aumailley M; Timpl R; Reisberg T; Tapiovaara H; Myohanen H; Murphy-Ullrich J; Vaheri A. (Department of Virology, University of Helsinki, Finland.) European journal of biochemistry / FEBS, (1992 Aug 1) 207 (3) 937-42. Journal code: 0107600. ISSN: 0014-2956. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Recently we have shown that heparin and related sulfated polyanions are low-affinity ligands of the kringle domain in the amino-terminal region (**ATF**) of human **urokinase** (u-PA), and proposed that this may facilitate loading of u-PA onto its receptor at the focal contacts between adherent cells and their matrix. We have now tested other components of the cell matrix (fibronectin, vitronectin, thrombospondin and laminin-nidogen) for u-PA binding, and found that laminin-nidogen is also a ligand of the u-PA **ATF**. Direct binding assays and competition binding assays with defined fragments of laminin-nidogen showed that there are u-PA binding sites in fragment E4 of laminin as well as in nidogen. The long-arm terminal domain of laminin (fragment E3), which contains a heparin-binding site, competed for binding of u-PA to immobilised heparin. However nidogen, which does not bind to heparin, also inhibited binding of u-PA to heparin, and this effect was also observed with recombinant nidogen and with a fragment of nidogen lacking the carboxy-terminal domain. Direct binding assays confirmed that u-PA binds to nidogen through a site in the u-PA **ATF**. We conclude that u-PA binds to laminin-nidogen by interactions involving the **ATF** region of u-PA, the E4 domain of laminin and the rod or amino-terminal regions of nidogen. Since nidogen is suggested to be an important bridging molecule in the maintenance of the supramolecular organization in basement membranes, the presence of a binding site for u-PA in nidogen indicates a role for plasminogen activation in basement membrane remodelling.

L15 ANSWER 110 OF 129 MEDLINE on STN

92340461. PubMed ID: 1376053. Purified alpha 2 macroglobulin receptor/LDL receptor-related protein binds **urokinase**.plasminogen activator inhibitor type-1 complex. Evidence that the alpha 2-macroglobulin receptor mediates cellular degradation of **urokinase** receptor-bound complexes. Nykjaer A; Petersen C M; Moller B; Jensen P H; Moestrup S K; Holtet T L; Etzerodt M; Thogersen H C; Munch M; Andreasen P A; +. (Institute of Medical Biochemistry, University of Aarhus, Denmark.) Journal of biological chemistry, (1992 Jul 25) 267 (21) 14543-6. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Complexes between 125I-labeled **urokinase**-type plasminogen activator (uPA) and plasminogen activator inhibitor type-1 (PAI-1) bound to purified alpha 2-macroglobulin (alpha 2M) receptor (alpha 2MR)/low density lipoprotein receptor-related protein (LRP). No binding was observed when using uPA. The magnitude of uPA.PAI-1 binding was comparable with that of the alpha 2MR-associated protein (alpha 2MRAP). Binding of uPA.PAI-1 was blocked by natural and recombinant alpha 2MRAP, and about 80% inhibited by complexes between tissue-type plasminogen activator (tPA) and PAI-1, and by a monoclonal anti-PAI-1 antibody. In human monocytes, uPA.PAI-1, like uPA and its **amino-terminal fragment**, bound to the **urokinase** receptor (uPAR). Degradation of uPAR-bound 125I-uPA.PAI-1 was 3-4-fold enhanced as compared with uncomplexed uPAR-bound uPA. The inhibitor-enhanced uPA degradation was blocked by r alpha 2MRAP and inhibited by polyclonal anti-alpha 2MR/LRP antibodies. This is taken as evidence for mediation of internalization and degradation of uPAR-bound uPA.PAI-1 by alpha 2MR/LRP.

L15 ANSWER 111 OF 129 MEDLINE on STN

92332522. PubMed ID: 1321137. Structural requirements for the growth factor activity of the amino-terminal domain of **urokinase**. Rabbani S A; Mazar A P; Bernier S M; Haq M; Bolivar I; Henkin J; Goltzman D. (Department of Medicine, Royal Victoria Hospital, McGill University, Montreal, Canada.) Journal of biological chemistry, (1992 Jul 15) 267 (20) 14151-6. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB High molecular weight **urokinase**-type plasminogen activator (uPA) in which proteolytic activity was inactivated (diisopropyl fluorophosphate (DFP)-uPA), its **amino-terminal fragment** (**ATF**, amino acids (aa) 1-143), and fucosylated and defucosylated growth factor domains (GFD, aa 4-43) were tested for growth-promoting effects and binding in human SaOS-2 osteosarcoma cells and U-937 lymphoma cells. DFP-uPA, **ATF**, and both the fucosylated and defucosylated GFD were capable of competing with 125I-**ATF** for binding to both SaOS-2 and U-937 cells. DFP-uPA, **ATF**, and fucosylated GFD were also mitogenic in SaOS-2 cells and increased cell numbers. However, defucosylated GFD was nonmitogenic in SaOS-2 cells and did not stimulate cell proliferation, even though it bound to these cells in a manner equivalent to the fucosylated GFD. A nonglycosylated high molecular weight uPA expressed and purified from Escherichia coli inhibited 125I-**ATF** binding to SaOS-2 cells but was also nonmitogenic. No mitogenic activity was observed in U-937 cells treated with the uPA forms capable of eliciting a mitogenic response in SaOS-2 cells. Proteolytically prepared kringle domain (aa 47-135) and low molecular weight uPA (aa 144-411) did not compete for 125I-**ATF** binding and did not elicit any mitogenic response in either of the cell lines tested. In addition, tissue plasminogen activator (tPA), which has been shown to be homologous to uPA in its growth factor domain and is also fucosylated, did not inhibit 125I-**ATF** binding nor elicit any mitogenic response. These results demonstrate that the GFD, implicated in binding to the uPA receptor, is also responsible for growth factor like activity in SaOS-2 cells and that the fucosylation at Thr18 within this domain may serve as a molecular trigger in eliciting this response.

L15 ANSWER 112 OF 129 MEDLINE on STN

92250509. PubMed ID: 1315748. Internalization of the **urokinase**-plasminogen activator inhibitor type-1 complex is mediated by the **urokinase** receptor. Olson D; Pollanen J; Hoyer-Hansen G; Ronne E; Sakaguchi K; Wun T C; Appella E; Dano K; Blasi F. (Institute of

Microbiology, University of Copenhagen, Denmark.) Journal of biological chemistry, (1992 May 5) 267 (13) 9129-33. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The role of the **urokinase** receptor (uPAR) in the internalization of the **urokinase**-plasminogen activator inhibitor type-1 (uPA.PAI-1) complex has been investigated. First, exploiting the species specificity of uPA binding, we show that mouse LB6 cells (that express a mouse uPAR) were unable to bind or degrade the human uPA.PAI-1 complex. On the other hand, LB6 clone 19 cells, which express a transfected human uPAR, degraded uPA.PAI-1 complexes with kinetics identical to the human monocytic U937 cells. We also show by immunofluorescence experiments with anti-uPA antibodies that in LB6 clone 19 cells, the uPA.PAI-1 complex is indeed internalized. While at 4 degrees C uPA fluorescence was visible at the cell surface, shift of the temperature to 37 degrees C caused a displacement of the immunoreactivity to the cytoplasmic compartment, with a pattern indicating lysosomal localization. If uPA.PAI-1 internalization/degradation is mediated by uPAR, inhibition of uPA.PAI-1 binding to uPAR should block degradation. Three different treatments, competition with the agonist **amino-terminal fragment** of uPA, treatment with a monoclonal antibody directed toward the binding domain of uPAR or release of uPAR from the cell surface with phosphatidylinositol-specific phospholipase C completely prevented uPA.PAI-1 degradation. The possibility that a serpin-enzyme complex receptor might be primarily or secondarily involved in the internalization process was excluded since a serpin-enzyme complex peptide failed to inhibit uPA.PAI-1 binding and degradation. Similarly, complexes of PAI-1 with low molecular mass uPA (33 kDa uPA), which lacks the uPAR binding domain, were neither bound nor degraded. Finally we also show that treatment of cells with uPA.PAI-1 complex caused a specific but partial down-regulation of uPAR. A similar result was obtained when PAI-1 was allowed to complex to uPA that had been previously bound to the receptor. The possibility therefore exists that the entire complex uPA.PAI-1-uPAR is internalized. All these data allow us to conclude that internalization of the uPA.PAI-1 complex is mediated by uPAR.

L15 ANSWER 113 OF 129 MEDLINE on STN

92210602. PubMed ID: 1313432. Demonstration of a specific clearance receptor for tissue-type plasminogen activator on rat Novikoff hepatoma cells. Nguyen G; Self S J; Camani C; Kruithof E K. (Division of Hematology, Centre Hospitalia Universitaire Vaudois, Lausanne, Switzerland.) Journal of biological chemistry, (1992 Mar 25) 267 (9) 6249-56. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The binding, internalization, and degradation of tissue-type plasminogen activator (t-PA) were studied in a rat hepatoma (Novikoff) cell line. Binding of t-PA to specific saturable high affinity binding sites (Kd = 12 nM, 54,000 sites/cell) was followed by internalization and degradation and did not require a functional active site. The catabolism of t-PA was not inhibited by an excess of **urokinase**-type plasminogen activator (u-PA), and t-PA bound to Novikoff membranes was not complexed to PAI-1, suggesting a mechanism independent of PAI-1. Additionally, a mannose receptor is not involved since t-PA binding was not influenced by an excess of mannose, galactose, ovalbumin, or EDTA. Furthermore, the degradation of t-PA was not influenced by 10 mM 6-aminohexanoic acid, a lysine analogue. The t-PA receptor binds to and can be eluted from wheat germ agglutinin-Sepharose. Cross-linking of t-PA with partially purified receptor and ligand blot analysis, suggest that t-PA binds to two proteins, a principal one of 55 kDa and a minor one of 43 kDa. Novikoff cells are able also to bind (Kd = 1.4 nM, 25,000 sites/cell) and degrade u-PA. The binding was inhibited by pro-u-PA and the **amino-terminal fragment** of u-PA, but not by an excess of t-PA. The u-PA receptor, but not the t-PA receptor, was removed by treatment with phosphatidylinositol-specific phospholipase C. Our results show that the clearance receptor for t-PA on Novikoff cells is different from the mannose receptor and the PAI-1-dependent receptor described in other cells. The rat hepatoma cells are thus a good model to study the PAI-1 independent hepatocyte-specific

clearance of t-PA.

L15 ANSWER 114 OF 129 MEDLINE on STN
92198395. PubMed ID: 1801751. Biological and clinical relevance of the **urokinase**-type plasminogen activator (uPA) in breast cancer. Schmitt M; Goretzki L; Janicke F; Calvete J; Eulitz M; Kobayashi H; Chucholowski N; Graeff H. (Frauenklinik, Technischen Universitat Munchen, Klinikum rechts der Isar, FRG.) Biomedica biochimica acta, (1991) 50 (4-6) 731-41. Journal code: 8304435. ISSN: 0232-766X. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.
AB Tumor cell invasion and metastasis is a multifactorial process, which at each step may require the action of proteolytic enzymes such as collagenases, cathepsins, plasmin, or plasminogen activators. An enzymatically inactive proenzyme form of the **urokinase**-type plasminogen activator (pro-uPA) is secreted by tumor cells which may be converted to an enzymatically active two-chain uPA-molecule (HMW-uPA) by plasmin-like enzymes. Action of proteases on pro-uPA may generate the enzymatically active or inactive high-molecular-weight form of uPA (HMW-uPA). Some proteases (plasmin, cathepsin B and L, kallikrein, trypsin or thermolysin) activate pro-uPA by cleaving the peptide bond Lys158 and IIe159. Other proteases (elastase, thrombin) cleave pro-uPA at different positions to yield enzymatically inactive HMW-uPA. HMW-uPA may be split into the enzymatically active LMW-uPA and the enzymatically inactive **ATF** (**amino terminal fragment**). **ATF** may be cleaved between peptide sequence 20 and 40 within the receptor binding domain of uPA (GFD). Such impaired **ATF** does not bind to uPA-receptors. Action of the bacterial endoproteinase Asp-N from Pseudomonas fragi mutant on pro-uPA or HMW-uPA, however, generates intact **ATF** which efficiently competes for binding of HMW-uPA or pro-uPA to receptors on tumor cells. High uPA-antigen content (pro-uPA, HMW-uPA, or LMW-uPA) in breast cancer tissue (not in plasma) indicates an elevated risk for the patient of recurrences and shorter overall survival. Thus pro-uPA/uPA-antigen content in breast cancer tissue serves as an independent prognostic parameter for the outcome of the disease. Cathepsin D is also an independent prognostic factor for recurrences and overall survival. High content of cathepsin D in breast cancer tumors is, however, not correlated with elevated levels of pro-uPA/uPA indicating that synthesis and release of cathepsin D and pro-uPA/uPA are independent events.

L15 ANSWER 115 OF 129 MEDLINE on STN
92129448. PubMed ID: 1734031. **Urokinase**-type plasminogen activator mediates basic fibroblast growth factor-induced bovine endothelial cell migration independent of its proteolytic activity. Odekon L E; Sato Y; Rifkin D B. (Department of Cell Biology, New York University Medical Center, New York.) Journal of cellular physiology, (1992 Feb) 150 (2) 258-63. Journal code: 0050222. ISSN: 0021-9541. Pub. country: United States. Language: English.
AB The dependence of **urokinase**-type plasminogen activator (uPA) induction on endogenous basic fibroblast growth factor (bFGF) activity during endothelial cell migration was investigated utilizing a combination of wounded endothelial cell monolayers and substrate overlay techniques. Purified polyclonal rabbit immunoglobulin G (IgG) against bFGF blocked the appearance of uPA-dependent lytic activity normally observed at the edge of a wounded bovine aortic endothelial (BAE) cell monolayer. Additionally, the migration of cells into the denuded area was inhibited 30-50% by antibodies either to bFGF or to bovine uPA. Incubation of wounded monolayers with either purified bovine uPA or agents able to induce PA activity, such as phorbol myristate acetate (PMA), vanadate, or bFGF, resulted in enhanced migration of cells (28-50%). Anti-bovine uPA IgG blocked a significant fraction (25%) of BAE cell migration induced by exposure to exogenous bFGF. The role of uPA in migration of wounded BAE cells was not dependent on plasmin generation. Furthermore, the **amino terminal fragment** (**ATF**) of human recombinant (hr) uPA, which is enzymatically inactive, stimulated endothelial cell movement in the presence of anti-bFGF IgG. These results suggest that BAE cell migration from the edge of a wounded monolayer is dependent upon local increases of

uPA mediated by endogenous bFGF. Moreover, the data support the conclusion that migration is stimulated via a signalling mechanism dependent upon occupancy of the uPA receptor but independent of uPA-mediated proteolysis.

L15 ANSWER 116 OF 129 MEDLINE on STN
91154379. PubMed ID: 1847936. An autocrine role for **urokinase** in phorbol ester-mediated differentiation of myeloid cell lines. Nusrat A R; Chapman H A Jr. (Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts 02115.) Journal of clinical investigation, (1991 Mar) 87 (3) 1091-7. Journal code: 7802877. ISSN: 0021-9738. Pub. country: United States. Language: English.
AB The human myeloid cell line HL60 secretes **urokinase**-type plasminogen activator (uPA) and expresses its receptor. When stimulated with phorbol myristate acetate (PMA), both secretion of uPA and the expression of its receptor are up-regulated, and these cells differentiate to an adherent phenotype. This adhesive response is markedly reduced in the presence of uPA antibodies. The PMA response is restored by the addition of native uPA, an **amino-terminal fragment** of uPA (residues 1-143) devoid of proteolytic activity, or a synthetic peptide (residues 12-32) from the uPA growth factor domain known to mediate receptor binding. In contrast, the addition of catalytically active low molecular weight uPA, which is missing the growth factor domain, or a peptide from the catalytic domain (residues 247-266) is ineffective. The influence of uPA antibodies on a second marker of macrophage differentiation, cysteine proteinase activity, was also examined. Cysteine proteinase activity of HL60 cells is increased in PMA-treated cells after 24 h but it fails to increase in the presence of anti-uPA. This increase in cathepsin B-like activity is also restored by exogenous uPA. These experiments indicate that an autocrine interaction of the growth factor domain of uPA with its receptor mediates an essential step in PMA-mediated myeloid cell differentiation.

L15 ANSWER 117 OF 129 MEDLINE on STN
91107700. PubMed ID: 1846368. Cellular receptor for **urokinase** plasminogen activator. Carboxyl-terminal processing and membrane anchoring by glycosyl-phosphatidylinositol. Ploug M; Ronne E; Behrendt N; Jensen A L; Blasi F; Dano K. (Finsen Laboratory, Rigshospitalet, Copenhagen, Denmark.) Journal of biological chemistry, (1991 Jan 25) 266 (3) 1926-33. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.
AB The cellular receptor for human **urokinase**-type plasminogen activator (u-PAR) is shown by several independent criteria to be a true member of a family of integral membrane proteins, anchored to the plasma membrane exclusively by a COOH-terminal glycosyl-phosphatidylinositol moiety. 1) Amino acid analysis of u-PAR after micropurification by affinity chromatography and N-[2-hydroxy-1,1-bis(hydroxymethyl)-ethyl]glycine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed the presence of 2-3 mol of ethanolamine/mol protein. 2) Membrane-bound u-PAR is efficiently released from the surface of human U937 cells by trace amounts of purified bacterial phosphatidylinositol-specific phospholipase C. This soluble form of u-PAR retains the binding specificity toward both u-PA and its **amino-terminal fragment** holding the receptor-binding domain. 3) Treatment of purified u-PAR with phosphatidylinositol-specific phospholipase C or mild alkali completely alters the hydrophobic properties of the receptor as judged by temperature-induced detergent-phase separation and charge-shift electrophoresis. 4) Biosynthetic labeling of u-PAR was obtained with [3H]ethanolamine and myo-[3H]inositol. 5) Finally, comparison of amino acid compositions derived from cDNA sequence and amino acid analysis shows that a polypeptide of medium hydrophobicity is excised from the COOH terminus of the nascent u-PAR. A similar proteolytic processing has been reported for other proteins that are linked to the plasma membrane by a glycosyl-phosphatidylinositol membrane anchor.

L15 ANSWER 118 OF 129 MEDLINE on STN
91097529. PubMed ID: 2125213. An **amino-terminal fragment** of

urokinase isolated from a prostate cancer cell line (PC-3) is mitogenic for osteoblast-like cells. Rabbani S A; Desjardins J; Bell A W; Banville D; Mazar A; Henkin J; Goltzman D. (Department of Physiology, McGill University, Montreal, Quebec, Canada.) Biochemical and biophysical research communications, (1990 Dec 31) 173 (3) 1058-64. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB A peptide mitogen for cultured osteoblast-like cells was purified from serum-free conditioned culture medium of a human prostatic cancer cell line, PC-3. Based on amino acid sequencing and estimated molecular weight, this peptide was identified as an NH2-terminal fragment of **urokinase**-type plasminogen activator (uPA). Recombinant high molecular weight (HMW) uPA and the NH2-terminal growth factor domain (GFD) of uPA, but not low molecular weight (LMW) uPA (lacking the NH2-terminal region) stimulated [3H] thymidine incorporation and proliferation in osteoblast-like cells, and specific, competitive binding sites for HMW, but not LMW, uPA were demonstrable. These studies demonstrate the production of a mitogenic NH2-terminal fragment of uPA by a human prostatic cancer cell line which may be of importance in the pathogenesis of osteoblastic metastases.

L15 ANSWER 119 OF 129 MEDLINE on STN

91083011. PubMed ID: 2175557. Alveolar macrophage **urokinase** receptors localize enzyme activity to the cell surface. Chapman H A; Bertozzi P; Sailor L Z; Nusrat A R. (Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts.) American journal of physiology, (1990 Dec) 259 (6 Pt 1) L432-8. Journal code: 0370511. ISSN: 0002-9513. Pub. country: United States. Language: English.

AB Human alveolar macrophages are known to synthesize **urokinase** (uPA) and a specific plasminogen activator inhibitor, PAI-2. In this study we have identified a uPA receptor expressed by these cells and defined the influence of PAI-2 on the interaction of uPA with its receptor. Alveolar macrophages from four normal volunteers were incubated with 55 kDa 125I-labeled uPA (0.24-8 nM) in the presence or absence of excess unlabeled uPA. Specific and saturable binding was demonstrable in all cases. Scatchard plots were linear; regression analysis revealed a mean Kd of 5.25 nM (range 3.2-6.7) and mean Bmax of 30.7 femtomoles/10(5) cells (range 21.5-34.5). The structure of the uPA receptor was defined by electroblotting membrane fractions of macrophages and sequentially exposing filters to uPA and uPA antibodies. Membranes from macrophages demonstrated binding of either uPA or a 15-kDa **amino-terminal fragment** of uPA to a 55- to 60-kDa glycosylated membrane protein. Binding of uPA to filters was blocked by a synthetic oligopeptide containing the known receptor binding region of native uPA. Preincubation of 125I-uPA with PAI-2 dramatically reduced the rate of association of uPA with macrophage uPA receptor. Conversely, receptor-bound uPA activity was less susceptible to inhibition by PAI-2 than soluble uPA activity. These data indicate that normal alveolar macrophages express uPA receptors. The receptor preferentially binds and protects free uPA over complexed enzyme, indicating that one function of the receptor is to allow the cells to express active uPA in an inhibitor-rich environment.

L15 ANSWER 120 OF 129 MEDLINE on STN

90214616. PubMed ID: 2157592. Receptor-mediated internalization and degradation of **urokinase** is caused by its specific inhibitor PAI-1. Cubellis M V; Wun T C; Blasi F. (Biotechnology Center for Molecular Cell Biology, University of Copenhagen, Denmark.) EMBO journal, (1990 Apr) 9 (4) 1079-85. Journal code: 8208664. ISSN: 0261-4189. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The receptor for **urokinase** plasminogen activator (uPA) has been previously shown not to internalize its ligand, but rather to focalize its activity at the cell surface, allowing a regulated cell surface plasmin dependent proteolysis. The receptor in fact binds the proenzyme pro-uPA and allows its very efficient conversion to the active two chains form. Receptor bound active uPA can also interact with its specific type 1 inhibiror (PAI-1) which is therefore able to inhibit the cell surface plasmin formation. In this paper we show that the uPA-PAI-1 complex bound

to the uPA receptor is internalized and degraded. U937 cells were incubated at 4 degrees C with labeled uPA-PAI-1 (and other ligands), the temperature then raised to 37 degrees C and the fate of the ligand followed for 3 h thereafter. The uPA-PAI-1 complex was internalized into the cells (i.e. could not be dissociated by acid treatment) and thereafter degraded (i.e. appeared in the supernatant in a non TCA-precipitable form). Other ligands (free uPA, **ATF** and DFP-treated uPA) were not internalized nor degraded. The degradation of the uPA-PAI-1 complex is preceded by internalization and is inhibited by chloroquine, an inhibitor of lysosomal protein degradation. These data suggest the existence of a cellular cycle of uPA. After synthesis pro-uPA is secreted, bound to the receptor and activated to two chain uPA. On the surface, uPA can activate surface bound plasminogen to produce surface bound plasmin. In the presence of PAI-1 uPA activity is inhibited and plasmin production interrupted, while the uPA-PAI-1 complex is internalized and degraded.

L15 ANSWER 121 OF 129 MEDLINE on STN

90202929. PubMed ID: 2156852. The human receptor for **urokinase** plasminogen activator. NH2-terminal amino acid sequence and glycosylation variants. Behrendt N; Ronne E; Ploug M; Petri T; Lober D; Nielsen L S; Schleuning W D; Blasi F; Appella E; Dano K. (Finsen Laboratory, Righospitalet, Copenhagen, Denmark.) Journal of biological chemistry, (1990 Apr 15) 265 (11) 6453-60. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The receptor for human **urokinase**-type plasminogen activator (u-PA) was purified from phorbol 12-myristate 13-acetate-stimulated U937 cells by temperature-induced phase separation of detergent extracts, followed by affinity chromatography with immobilized diisopropyl fluorophosphate-treated u-PA. The purified protein shows a single 55-60 kDa band after sodium dodecyl sulfate-polyacrylamide gel electrophoresis and silver staining. It is a heavily glycosylated protein, the deglycosylated polypeptide chain comprising only 35 kDa. The glycosylated protein contains N-acetyl-D-glucosamine and sialic acid, but no N-acetyl-D-galactosamine. Glycosylation is responsible for substantial heterogeneity in the receptor on phorbol ester-stimulated U937 cells, and also for molecular weight variations among various cell lines. The amino acid composition and the NH2-terminal amino acid sequence are reported. The protein has a high content of cysteine residues. The NH2-terminal sequence is not closely related to any known sequence. The identification of the purified and sequenced protein with the human u-PA receptor is based on the following findings: 1) the ability of the purified protein to bind u-PA and its **amino-terminal fragment**; 2) the identical electrophoretic mobilities observed for cross-linked conjugates, formed between either the purified protein or the u-PA receptor on intact U937 cells and the above ligands; 3) the identity of the apparent molecular weight of the purified protein to that predicted for the u-PA receptor in the same cross-linking studies; 4) the identical extent of glycosylation of the purified protein and of the u-PA receptor in crude membrane fractions, as detected after cross-linking; 5) the ability of antibodies raised against the purified protein to inhibit cellular binding of the **amino-terminal fragment** of u-PA.

L15 ANSWER 122 OF 129 MEDLINE on STN

90201283. PubMed ID: 2156717. Localization of **urokinase**-type plasminogen activator receptor on U937 cells: phorbol ester PMA induces heterogeneity. Hansen S H; Behrendt N; Dano K; Kristensen P. (Finsen Laboratory, Rigshospitalet, Copenhagen, Denmark.) Experimental cell research, (1990 Apr) 187 (2) 255-62. Journal code: 0373226. ISSN: 0014-4827. Pub. country: United States. Language: English.

AB The binding of human **urokinase**-type plasminogen activator (u-PA) to the surface of the human monocytic cell line U937 was studied by immunological detection of bound u-PA or binding of biotinylated diisopropyl fluorophosphate-inactivated human u-PA visualized by light or electron microscopy. Untreated U937 cells showed a characteristic binding pattern, with the majority of the u-PA bound to the microvillar-containing protruding pole of the cells. After treatment with the phorbol ester PMA,

the resulting adherent cell population was very heterogeneous with respect to both cellular morphology and u-PA binding. The bound u-PA was distributed on both the dorsal and the substrate side of the cells, and the patches of bound u-PA could not be correlated to any typical membrane conformations or cell-cell or cell-substratum contacts. When a monoclonal antibody directed against the **amino-terminal fragment** (**ATF**) of u-PA was used, the results were identical regardless of whether intact u-PA or **ATF** was used for binding to the cells. In contrast, when a monoclonal antibody recognizing the non-receptor-binding protease domain of u-PA was used, bound **ATF** showed no staining, while bound intact u-PA was stained as efficiently as above. The alteration of u-PA receptor distribution following treatment with PMA could be related to the changes in glycosylation and ligand affinity of the purified u-PA receptor previously described following PMA treatment of U937 cells.

L15 ANSWER 123 OF 129 MEDLINE on STN

90153917. PubMed ID: 2154462. Interaction of single-chain **urokinase**-type plasminogen activator with human endothelial cells. Barnathan E S; Kuo A; Rosenfeld L; Kariko K; Leski M; Robbiati F; Nolli M L; Henkin J; Cines D B. (Department of Medicine, University of Pennsylvania, School of Medicine, Philadelphia 19104.) Journal of biological chemistry, (1990 Feb 15) 265 (5) 2865-72. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The interaction of **urokinase**-type plasminogen activators with receptors on the surface of endothelial cells may play an important role in the regulation of fibrinolysis and cell migration. Therefore, we investigated whether human umbilical vein endothelial cells (HUVEC) express receptors for single-chain **urokinase** (scu-PA) on the cell surface and examined the effect of such binding on plasminogen activator activity. Binding of 125I-labeled scu-PA to HUVEC, performed at 4 degrees C, was saturable, reversible, and specific (k+1 4 +/- 1 X 10(6) min-1 M-1, k-1 6.2 +/- 1.4 X 10(-3) min-1, Kd 2.8 +/- 0.1 nM; Bmax 2.2 +/- 0.1 X 10(5) sites/cell; mean +/- S.E.). Binding of radiolabeled scu-PA was inhibited by both natural and recombinant wild-type scu-PA, high molecular weight two-chain u-PA (tcu-PA), catalytic site-inactivated tcu-PA, an **amino-terminal fragment** of u-PA (amino acids 1-143), and a smaller peptide (amino acids 4-42) corresponding primarily to the epidermal growth factor-like domain. Binding was not inhibited by low molecular weight **urokinase** or by a recombinant scu-PA missing amino acids 9-45. Cell-bound scu-PA migrated at its native molecular mass on sodium dodecyl sulfate-polyacrylamide gel electrophoresis. In the presence of plasminogen, scu-PA bound to endothelial cells generated greater plasmin activity than did scu-PA in the absence of cells. In contrast, when tcu-PA was added directly to HUVEC, sodium dodecyl sulfate-stable complexes formed with cell or matrix-associated plasminogen activator inhibitors with a loss of plasminogen activator activity. These studies suggest that endothelial cells in culture express high affinity binding sites for the epidermal growth factor domain of scu-PA. Interaction of scu-PA with these receptors may permit plasminogen activator activity to be expressed at discrete sites on the endothelial cell membrane.

L15 ANSWER 124 OF 129 MEDLINE on STN

90085165. PubMed ID: 2480654. Epitope mapping of the anti-**urokinase** monoclonal antibody 5B4 by isolated domains of **urokinase**. Corti A; Sarubbi E; Soffientini A; Nolli M L; Zanni A; Galimberti M; Parenti F; Cassani G. (Merrell Dow Research Institute, Lepetit Research Center, Gerenzano (Varese), Italy.) Thrombosis and haemostasis, (1989 Nov 24) 62 (3) 934-9. Journal code: 7608063. ISSN: 0340-6245. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.

AB The **amino terminal fragment** (**ATF**) of **urokinase**-type plasminogen activator (uPA) is a degradation product comprising the entire growth factor-like and kringle domains. It has been previously shown that **ATF** is able to bind to the u-PA receptor through the growth factor-like domain and that the anti u-PA monoclonal antibody 5B4 (Mab 5B4) binds to **ATF** preventing u-PA receptor binding. To localize more precisely the epitope recognized by Mab 5B4, **ATF** was subfragmented by controlled enzymatic

proteolysis with V8 protease. Three subfragments of 4,000 Mr (F-4k), 11,000 Mr (F-11k) and 12,000 Mr (F-12k) were purified from the reaction mixture and characterized. SDS-PAGE under reducing and non-reducing conditions, N-terminal aminoacid sequence analysis and C-terminal aminoacid analysis of each fragment indicate that F-4k and F-11k correspond to intact growth factor-like domain and kringle domain (residues 4-43 and 44-135 respectively) while F-12k corresponds to the kringle domain cleaved in the first loop at the glu52-gly53 bond. By Western blot and competitive binding experiments we show that Mab 5B4 recognizes an epitope located on the kringle domain of u-PA and that the binding is strongly reduced when the kringle contains an additional cleavage in its first loop. Since the receptor binding site of u-PA has been previously shown to be located on the growth factor-like domain, Mab 5B4 inhibits the binding of uPA to its cellular receptor likely by steric hindrance. Besides the proven utility in epitope localization of anti u-PA monoclonal antibodies, these u-PA fragments may represent powerful tools for studies of structure-function relationship of u-PA.

L15 ANSWER 125 OF 129 MEDLINE on STN

89296897. PubMed ID: 2544876. Accessibility of receptor-bound **urokinase** to type-1 plasminogen activator inhibitor. Cubellis M V; Andreasen P; Ragno P; Mayer M; Dano K; Blasi F. (Institute of Microbiology, University of Copenhagen, Denmark.) Proceedings of the National Academy of Sciences of the United States of America, (1989 Jul) 86 (13) 4828-32. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB **Urokinase** plasminogen activator (uPA) interacts with a surface receptor and with specific inhibitors, such as plasminogen activator inhibitor type 1 (PAI-1). These interactions are mediated by two functionally independent domains of the molecule: the catalytic domain (at the carboxyl terminus) and the growth factor domain (at the amino terminus). We have now investigated whether PAI-1 can bind and inhibit receptor-bound uPA. Binding of 125I-labeled **ATF (amino-terminal fragment** of uPA) to human U937 monocyte-like cells can be competed for by uPA-PAI-1 complexes, but not by PAI-1 alone. Performed 125I-labeled uPA-PAI-1 complexes can bind to uPA receptor with the same binding specificity as uPA. PAI-1 also binds to, and inhibits the activity of, receptor-bound uPA in U937 cells, as shown in U937 cells by a caseinolytic plaque assay. Plasminogen activator activity of these cells is dependent on exogenous uPA, is competed for by receptor-binding diisopropyl fluorophosphate-treated uPA, and is inhibited by the addition of PAI-1. In conclusion, in U937 cells the binding to the receptor does not shield uPA from the action of PAI-1. The possibility that in adherent cells a different localization of PAI-1 and uPA leads to protection of uPA from PAI-1 is to be considered.

L15 ANSWER 126 OF 129 MEDLINE on STN

89123287. PubMed ID: 2521625. Plasminogen activation initiated by single-chain **urokinase**-type plasminogen activator. Potentiation by U937 monocytes. Ellis V; Scully M F; Kakkar V V. (Thrombosis Research Unit, King's College School of Medicine and Dentistry, London, United Kingdom.) Journal of biological chemistry, (1989 Feb 5) 264 (4) 2185-8. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The binding of **urokinase**-type plasminogen activators (u-PA) to receptors on various cell types has been proposed to be an important feature of many cellular processes requiring extracellular proteolysis. We have investigated the effect of single-chain u-PA binding to the monocyte-like cell line U937 on plasminogen activation. A 16-fold acceleration of the activation of plasminogen was observed at optimal concentrations of single-chain u-PA. This potentiation was abolished by the addition of either 6-aminohexanoic acid or the **amino-terminal fragment** of u-PA, thus demonstrating the requirement for specific binding of both single-chain u-PA and plasminogen to the cells. The mechanism of the enhancement of plasmin generation appears to be due primarily to an increase in the rate of feedback activation of single-chain u-PA to the more active two-chain u-PA by cell-bound plasmin, initially generated by

single chain u-PA. This increased activity of the plasminogen activation system in the presence of U937 cells provides a mechanism whereby u-PAs may exert their influence in a variety of cell-associated proteolytic events.

L15 ANSWER 127 OF 129 MEDLINE on STN

88115381. PubMed ID: 2828365. A 55,000-60,000 Mr receptor protein for **urokinase**-type plasminogen activator. Identification in human tumor cell lines and partial purification. Nielsen L S; Kellerman G M; Behrendt N; Picone R; Dano K; Blasi F. (Finsen Laboratory, Rigshospitalet, Copenhagen, Denmark.) Journal of biological chemistry, (1988 Feb 15) 263 (5) 2358-63. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The iodinated Mr approximately equal to 15,000 **amino-terminal fragment** (**ATF**) of the **urokinase**-type plasminogen activator (u-PA) molecule bound specifically to the cell surface of all of seven cultured human tumor cell lines studied. Cross-linking of iodinated **ATF** to the cell surface using a bifunctional amino-reactive reagent followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography revealed with the four cell lines studied the occurrence of a single band migrating with an Mr of 70,000-75,000, indicating complex formation with an Mr of 55,000-60,000 u-PA receptor protein (u-PA-R). In the human monocyte cell line U937 cultivated in the presence of phorbol ester, the amount of complex was strongly increased, and a fraction of the complex had a slower electrophoretic mobility. Comparison between autoradiograms of reduced and unreduced samples suggests that u-PA-R consists of one polypeptide chain. Two forms of u-PA-R, which differed with respect to affinity to concanavalin A, were identified. u-PA-R retained its ability to bind to **ATF** after cell lysis, and it was purified approximately 2,200-fold from biosynthetically labeled U937 cells by affinity chromatography with proenzyme u-PA coupled to Sepharose. The purified Mr 55,000-60,000 protein was specifically bound and cross-linked to u-PA, proenzyme u-PA, and **ATF**, but not to tissue-type plasminogen activator or other unrelated proteins.

L15 ANSWER 128 OF 129 MEDLINE on STN

88089216. PubMed ID: 3121772. Sensitive and specific enzyme-linked immunosorbent assay for **urokinase**-type plasminogen activator and its application to plasma from patients with breast cancer. Grondahl-Hansen J; Agerlin N; Munkholm-Larsen P; Bach F; Nielsen L S; Dombernowsky P; Dano K. (Finsen Laboratory, Rigshospitalet, Copenhagen, Denmark.) Journal of laboratory and clinical medicine, (1988 Jan) 111 (1) 42-51. Journal code: 0375375. ISSN: 0022-2143. Pub. country: United States. Language: English.

AB An enzyme-linked immunosorbent assay (ELISA) was developed for the measurement of human **urokinase**-type plasminogen activator (u-PA) in plasma and serum. Microtiter plates were coated with a monoclonal antibody and incubated with standard or sample. Bound u-PA was quantitated with polyclonal antibodies conjugated with biotin, followed by avidin-peroxidase. The assay was 10 times as sensitive as previously reported immunoassays, the detection limit being approximately 1 pg u-PA in a volume of 100 microliter, with a linear dose-response up to 15 pg u-PA. The assay detected active u-PA and its inactive proenzyme form equally well, and the recovery of both forms was higher than 90% in plasma. It also detected u-PA complexed with plasminogen activator inhibitor type 1. Various structurally related proteins, including t-PA, were tested, but no reaction was observed with proteins other than u-PA and its **amino-terminal fragment**. The intra-assay and interassay coefficients of variation for determination of u-PA in plasma were 7.6% and 8.4%, respectively. The ELISA was used to measure the concentration of u-PA in plasma from 34 healthy donors and 92 patients with breast cancer with a varying extent of disease. The mean value for the healthy donors was 1.1 +/- 0.3 ng/ml (SD) of u-PA in plasma. This value is substantially lower than those previously reported. The mean value for the patients with breast cancer was 1.3 +/- 0.4 ng/ml. This moderate increase was statistically significant at the 1% level. Approximately one quarter of the patients had plasma u-PA concentrations above the range

observed for the healthy controls. There was a positive correlation between the mean u-PA plasma concentration and the extent of disease in different groups of patients.

L15 ANSWER 129 OF 129 MEDLINE on STN

87165839. PubMed ID: 3031025. The receptor-binding sequence of **urokinase**. A biological function for the growth-factor module of proteases. Appella E; Robinson E A; Ullrich S J; Stoppelli M P; Corti A; Cassani G; Blasi F. Journal of biological chemistry, (1987 Apr 5) 262 (10) 4437-40. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Previous studies have shown that the region of human **urokinase**-type plasminogen activator (uPA) responsible for receptor binding resides in the **amino-terminal fragment** (**ATF**, residues 1-135) (Stoppelli, M.P., Corti, A., Soffientini, A., Cassani, G., Blasi, F., and Assoian, R.K. (1985) Proc. Natl. Acad. Sci. U.S. A. 82, 4939-4943). The area within **ATF** responsible for specific receptor binding has now been identified by the ability of different synthetic peptides corresponding to different regions of the amino terminus of uPA to inhibit receptor binding of 125I-labeled **ATF**. A peptide corresponding to human [Ala19]uPA-(12-32) resulted in 50% inhibition of **ATF** binding at 100 nM. Peptides uPA-(18-32) and [Ala13]uPA-(9-20) inhibit at 100 and 2000 microM, respectively. The human peptide uPA-(1-14) and the mouse peptide [Ala20]uPA-(13-33) have no effect on **ATF** receptor binding. This region of uPA is referred to as the growth factor module since it shares partial amino acid sequence homology (residues 14-33) to epidermal growth factor (EGF). Furthermore, this region of EGF is responsible for binding of EGF to its receptor (Komoriya, A. Hortsch, M., Meyers, C., Smith, M., Kanety, H., and Schlessinger, J. (1984) Proc. Natl. Acad. Sci. U.S.A. 81, 1351-1355). However, EGF does not inhibit **ATF** receptor binding. Comparison of the sequences responsible for receptor binding of uPA and EGF indicate that the region of highest homology is between residues 13-19 and 14-20 of human uPA and EGF, respectively. In addition, there is a conservation of the spacings of four cysteines in this module whereas there is no homology between residues 20-30 and 21-33 of uPA and EGF. Thus, residues 20-30 of uPA apparently confer receptor binding specificity, and residues 13-19 provide the proper conformation to the adjacent binding region.

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(FILE 'HOME' ENTERED AT 22:23:33 ON 10 MAR 2004)

FILE 'USPATFULL' ENTERED AT 22:24:23 ON 10 MAR 2004

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              E WADA MANABU/IN
L1            7 S E3
              E WADA NAOKO/IN
L2            2 S E3
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FILE 'MEDLINE' ENTERED AT 22:25:28 ON 10 MAR 2004

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              E WADA M/AU
L3          875 S E3
L4            2 S L3 AND (UROKINASE)
              E WADA N/AU
L5            3 S E10
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FILE 'USPATFULL' ENTERED AT 22:28:52 ON 10 MAR 2004

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L6         6244 S UROKINASE
L7           38 S L6 AND (HUMAN UROKINASE PLASMINOGEN ACTIVATOR)
L8            2 S L7 AND (HUMAN UROKINASE PLASMINOGEN ACTIVATOR/CLM)
L9           36 S L7 NOT L8
L10         166 S L6 AND (ATF OR AMINO-TERMINAL FRAGMENT)
L11          13 S L10 AND (ATF/CLM OR AMINO-TERMINAL FRAGMENT/CLM)
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FILE 'MEDLINE' ENTERED AT 22:38:16 ON 10 MAR 2004

L12 3092 S (UROKINASE OR HUMAN UROKINASE PLASMINOGEN ACTIVATOR)
L13 26 S L12 AND (HUMAN UROKINASE PLASMINOGEN ACTIVATOR)
L14 133 S L12 AND (ATF OR AMINO TERMINAL FRAGMENT)
L15 129 S L14 NOT L13

=> s l12 and (HIV or human immunodeficiency virus)
134513 HIV
8435981 HUMAN
112146 IMMUNODEFICIENCY
370197 VIRUS
42307 HUMAN IMMUNODEFICIENCY VIRUS
(HUMAN(W)IMMUNODEFICIENCY(W)VIRUS)
L16 26 L12 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)

=> d l16,cbib,1-26

L16 ANSWER 1 OF 26 MEDLINE on STN
2004093355. PubMed ID: 14982725. **Human immunodeficiency virus** type 1 Tat and methamphetamine affect the release and activation of matrix-degrading proteinases. Conant Katherine; St Hillaire Coryse; Anderson Caroline; Galey David; Wang Jessica; Nath Avindra. (Department of Neurology, Johns Hopkins University, Baltimore, Maryland, USA.) Journal of neurovirology, (2004 Feb) 10 (1) 21-8. Journal code: 9508123. ISSN: 1355-0284. Pub. country: United States. Language: English.

L16 ANSWER 2 OF 26 MEDLINE on STN
2003517119. PubMed ID: 12960238. The role of **urokinase**-type plasminogen activator (uPA)/uPA receptor in **HIV**-1 infection. Alfano Massimo; Sidenius Nicolai; Blasi Francesco; Poli Guido. (Department of Immunology and Infectious Disease, Vita-Salute University School of Medicine, Milan, Italy.) Journal of leukocyte biology, (2003 Nov) 74 (5) 750-6. Ref: 82. Journal code: 8405628. ISSN: 0741-5400. Pub. country: United States. Language: English.

L16 ANSWER 3 OF 26 MEDLINE on STN
2002401712. PubMed ID: 12150480. The serum level of soluble **urokinase** receptor is elevated in tuberculosis patients and predicts mortality during treatment: a community study from Guinea-Bissau. Eugen-Olsen J; Gustafson P; Sidenius N; Fischer T K; Parner J; Aaby P; Gomes V F; Lisse I. (Clinical Research Unit, Hvidovre Hospital, Denmark.. jeo@biobase.dk) . international journal of tuberculosis and lung disease : official journal of the International Union against Tuberculosis and Lung Disease, (2002 Aug) 6 (8) 686-92. Journal code: 9706389. ISSN: 1027-3719. Pub. country: France. Language: English.

L16 ANSWER 4 OF 26 MEDLINE on STN
2002342365. PubMed ID: 12084931. **Urokinase-urokinase** receptor interaction mediates an inhibitory signal for **HIV**-1 replication. Alfano Massimo; Sidenius Nicolai; Panzeri Barbara; Blasi Francesco; Poli Guido. (AIDS Immunopathogenesis Unit, Department of Immunology and Infectious Diseases, San Raffaele Scientific Institute, Via Olgettina n.58, 20132 Milan, Italy.) Proceedings of the National Academy of Sciences of the United States of America, (2002 Jun 25) 99 (13) 8862-7. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

L16 ANSWER 5 OF 26 MEDLINE on STN
2002240034. PubMed ID: 11967123. Decreased **urokinase** receptor expression on granulocytes in **HIV**-infected patients. Storgaard M; Obel N; Black F T; Moller B K. (Department of Infectious Diseases, Skejby University Hospital, Denmark.. m.storgaard@dadlnet.dk) . Scandinavian journal of immunology, (2002 Apr) 55 (4) 409-13. Journal code: 0323767. ISSN: 0300-9475. Pub. country: England: United Kingdom. Language: English.

L16 ANSWER 6 OF 26 MEDLINE on STN
2002173337. PubMed ID: 11906036. **Urokinase** plasminogen activator and TGF-beta production in immunosuppressed patients with and without

Pneumocystis carinii. Angelici E; Concini C; Cariagna F; Romani R; Magno M S; Serra P; Canipari R. (Department of Clinical Medicine, University La Sapienza, Rome, Italy.. Elena.angelici@uniroma1.it) . Journal of eukaryotic microbiology, (2001) Suppl 150S-151S. Journal code: 9306405. ISSN: 1066-5234. Pub. country: United States. Language: English.

L16 ANSWER 7 OF 26 MEDLINE on STN
2001347761. PubMed ID: 11409912. Exogenous fibroblast growth factor-2 induces a transformed phenotype in vascular kaposi's sarcoma-like cells. Cavallaro U; Soria M R; Montesano R. (Dibit, Scientific Institute San Raffaele, Milan, Italy.. cavallaro@nt.imp.univie.ac.at) . Molecular cell biology research communications : MCBRC, (2000 Oct) 4 (4) 203-5. Journal code: 100889076. ISSN: 1522-4724. Pub. country: United States. Language: English.

L16 ANSWER 8 OF 26 MEDLINE on STN
2001327647. PubMed ID: 11394884. Amino-terminal fragment of **urokinase**-type plasminogen activator inhibits **HIV**-1 replication. Wada M; Wada N A; Shirono H; Taniguchi K; Tsuchie H; Koga J. (Laboratories for Bioengineering and Research, JCR Pharmaceuticals Company, Ltd., 2-2-10 Murotani, Nishi-ku, Kobe, 651-2241, Japan.. wada-m@jcrpharm.co.jp) . Biochemical and biophysical research communications, (2001 Jun 8) 284 (2) 346-51. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

L16 ANSWER 9 OF 26 MEDLINE on STN
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L16 ANSWER 10 OF 26 MEDLINE on STN
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L16 ANSWER 11 OF 26 MEDLINE on STN
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L16 ANSWER 12 OF 26 MEDLINE on STN
1998380991. PubMed ID: 9715259. Altered levels of **urokinase** on monocytes and in serum of children with AIDS; effects on lymphocyte activation and surface marker expression. Murali R; Wolfe J H; Erber R; Chice S M; Murali M R; Durkin H G; Zach P; Auci D L. (Department of Pathology, State University of New York Health Science Center at Brooklyn, NY 11203, USA.) Journal of leukocyte biology, (1998 Aug) 64 (2) 198-202. Journal code: 8405628. ISSN: 0741-5400. Pub. country: United States. Language: English.

L16 ANSWER 13 OF 26 MEDLINE on STN
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J, Maier J A, Soria M R. (Department of Biological and Technological Research, San Raffaele Scientific Institute, Milano, Italy.. cavallu@dibit.hsr.it) . FASEB journal : official publication of the Federation of American Societies for Experimental Biology, (1998 Aug) 12 (11) 1027-34. Journal code: 8804484. ISSN: 0892-6638. Pub. country: United States. Language: English.

L16 ANSWER 14 OF 26 MEDLINE on STN
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L16 ANSWER 15 OF 26 MEDLINE on STN
97288653. PubMed ID: 9143604. Upregulation of **urokinase**-type plasminogen activator by endogenous and exogenous **HIV**-1 Tat protein in tumour cell lines derived from BK virus/tat-transgenic mice. Rusnati M; Coltrini D; Campioni D; Tanghetti E; Corallini A; Barbanti-Brodano G; Giuliani R; Gibellini D; Presta M. (Department of Biomedical Sciences and Biotechnology, University of Brescia, Italy.) AIDS (London, England), (1997 May) 11 (6) 727-36. Journal code: 8710219. ISSN: 0269-9370. Pub. country: United States. Language: English.

L16 ANSWER 16 OF 26 MEDLINE on STN
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L16 ANSWER 17 OF 26 MEDLINE on STN
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L16 ANSWER 18 OF 26 MEDLINE on STN
96256755. PubMed ID: 8676469. A role for **urokinase**-type plasminogen activator in **human immunodeficiency virus** type 1 infection of macrophages. Handley M A; Steigbigel R T; Morrison S A. (Department of Pharmacology, University Medical Center at Stony Brook, Stony Brook, New York, USA.) Journal of virology, (1996 Jul) 70 (7) 4451-6. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

L16 ANSWER 19 OF 26 MEDLINE on STN
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L16 ANSWER 20 OF 26 MEDLINE on STN
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Hansen J K, Guerrini L, Blasi F. (Department of Genetics and Microbiol Biology, University of Milano, Italy.) Journal of biological chemistry, (1994 Sep 2) 269 (35) 22230-7. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

L16 ANSWER 21 OF 26 MEDLINE on STN
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L16 ANSWER 22 OF 26 MEDLINE on STN
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L16 ANSWER 23 OF 26 MEDLINE on STN
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L16 ANSWER 24 OF 26 MEDLINE on STN
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L16 ANSWER 25 OF 26 MEDLINE on STN
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L16 ANSWER 1 OF 26 MEDLINE on STN
2004093355. PubMed ID: 14982725. **Human immunodeficiency virus** type 1 Tat and methamphetamine affect the release and activation of matrix-degrading proteinases. Conant Katherine; St Hillaire Coryse; Anderson Caroline; Galey David; Wang Jessica; Nath Avindra. (Department of Neurology, Johns Hopkins University, Baltimore, Maryland, USA.) Journal

of neurovirology, (2004 Feb) 10 (1) 21-6. Journal code: 9500125. ISSN: 1355-0284. Pub. country: United States. Language: English.

AB **Human immunodeficiency virus** (**HIV**) dementia (HIVD) is associated with an increase in the number of activated monocytes within the central nervous system (CNS), a pathological feature that may be more remarkable in the setting of superimposed substance abuse. Monocytes may transport **HIV** to the brain, and, moreover, activated and/or infected monocytes have been shown to release a number of potent neurotoxins. Although the mechanisms responsible for the increase in the CNS ingress of monocytes are multiple, blood-brain barrier (BBB)-degrading matrix metalloproteinases (MMPs) are likely to play an important role. The current study investigates the effects of the **HIV**-1-encoded protein Tat, and the drug of abuse methamphetamine, on MMP release from brain derived cells. The release of **urokinase** plasminogen activator (uPA), an activator of MMPs, was also investigated. Mixed human neuron/astrocyte cultures were stimulated with Tat or methamphetamine, and supernatants were analyzed by enzyme-linked immunosorbent assay (ELISA) and/or gelatin substrate zymography. Results showed that Tat and methamphetamine increased the release of MMP-1 from these cultures. Tat also increased supernatant levels of active MMP-2. In addition, both Tat and methamphetamine stimulated the release of the MMP activator uPA, and in a manner that was sensitive to inhibition with pertussis toxin. Together, these results suggest that in HIVD, Tat and methamphetamine may contribute to CNS inflammation by stimulating increased release and/or activation of matrix-degrading proteinases through mechanisms that include Gi/Go-coupled signaling. These results also suggest a potential mechanism for acceleration of HIVD with methamphetamine use.

L16 ANSWER 2 OF 26 MEDLINE on STN

2003517119. PubMed ID: 12960238. The role of **urokinase**-type plasminogen activator (uPA)/uPA receptor in **HIV**-1 infection. Alfano Massimo; Sidenius Nicolai; Blasi Francesco; Poli Guido. (Department of Immunology and Infectious Disease, Vita-Salute University School of Medicine, Milan, Italy.) Journal of leukocyte biology, (2003 Nov) 74 (5) 750-6. Ref: 82. Journal code: 8405628. ISSN: 0741-5400. Pub. country: United States. Language: English.

AB The binding of **urokinase**-type plasminogen activator (uPA) to its glycosyl-phosphatidyl-inositol (GPI) anchored receptor (uPAR) mediates a variety of functions in terms of vascular homeostasis, inflammation and tissue repair. Both uPA and uPAR, as well as their soluble forms detectable in plasma and other body fluids, represent markers of cancer development and metastasis, and they have been recently described as predictors of **human immunodeficiency virus** (**HIV**) disease progression, independent of CD4+ T cell counts and viremia. A direct link between the uPA/uPAR system and **HIV** infection was earlier proposed in terms of cleavage of gp120 envelope by uPA. More recently, a negative regulatory effect on both acutely and chronically infected cells has been linked to the noncatalytic portion of uPA, also referred to as the amino-terminal fragment (ATF). ATF has also been described as a major CD8+ T cell soluble **HIV** suppressor factor. In chronically infected promonocytic U1 cells this inhibitory effect is exerted at the very late stages of the virus life cycle, involving virion budding and entrapment in intracytoplasmic vacuoles, whereas its mechanism of action in acutely infected cells remains to be defined. Since uPAR is a GPI-anchored receptor it requires association with a signaling-transducing component and different partners, which include CD11b/CD18 integrin and a G-protein coupled receptor homologous to that for the bacterial chemotactic peptide formyl-methionyl-leucyl-phenylalanine. Which signaling coreceptor(s) is(are) responsible for uPA-dependent anti-**HIV** effect remains currently undefined.

L16 ANSWER 3 OF 26 MEDLINE on STN

2002401712. PubMed ID: 12150480. The serum level of soluble **urokinase** receptor is elevated in tuberculosis patients and predicts mortality during treatment: a community study from Guinea-Bissau. Eugen-Olsen J; Gustafson P; Sidenius N; Fischer T K; Parner J; Aaby P; Gomes V F; Lisse

1. (Clinical Research Unit, Hvidovre Hospital, Denmark.. jeo@biobase.dk) . international journal of tuberculosis and lung disease : official journal of the International Union against Tuberculosis and Lung Disease, (2002 Aug) 6 (8) 686-92. Journal code: 9706389. ISSN: 1027-3719. Pub. country: France. Language: English.

AB OBJECTIVE: To investigate whether the serum level of soluble **urokinase** plasminogen activator receptor (suPAR) carries prognostic information in individuals infected with Mycobacterium tuberculosis. DESIGN: suPAR was measured by ELISA in 262 individuals at the time of enrolment into a cohort based on suspicion of active tuberculosis and in 101 individuals after 8 months of follow-up. RESULTS: The suPAR levels were elevated in patients with active TB compared to TB-negative individuals ($P < 0.001$). suPAR levels were highest in patients positive for TB on direct microscopy ($n = 84$, median suPAR 3.17 ng/ml, $P < 0.001$), followed by patients negative on direct microscopy but culture positive ($n = 35$, median suPAR 2.41 ng/ml, $P = 0.005$) and by patients diagnosed on clinical grounds ($n = 63$, median suPAR 2.13 ng/ml, $P = 0.06$) compared to 64 TB-negative individuals (median suPAR 1.73 ng/ml). During the 8-month treatment period, 23 TB cases died. In a multivariate Cox model controlling for **HIV** status, age, sex, CD4 count and type of TB diagnosis, the mortality increase per ng suPAR was 1.25 (95%CI 1.12-1.40). After treatment, suPAR levels had decreased to the levels of TB-negative individuals. CONCLUSIONS: suPAR levels are elevated in TB patients and associated with mortality. Furthermore, suPAR may be a potential marker of treatment efficacy.

L16 ANSWER 4 OF 26 MEDLINE on STN

2002342365. PubMed ID: 12084931. **Urokinase-urokinase** receptor interaction mediates an inhibitory signal for **HIV**-1 replication. Alfano Massimo; Sidenius Nicolai; Panzeri Barbara; Blasi Francesco; Poli Guido. (AIDS Immunopathogenesis Unit, Department of Immunology and Infectious Diseases, San Raffaele Scientific Institute, Via Olgettina n.58, 20132 Milan, Italy.) Proceedings of the National Academy of Sciences of the United States of America, (2002 Jun 25) 99 (13) 8862-7. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB Elevated levels of soluble **urokinase**-type plasminogen activator (uPA) receptor, CD87/u-PAR, predict survival in individuals infected with **HIV**-1. Here, we report that pro-uPA (or uPA) inhibits **HIV**-1 expression in U937-derived chronically infected promonocytic U1 cells stimulated with phorbol 12-myristate 13-acetate (PMA) or tumor necrosis factor-alpha (TNF-alpha). However, pro-uPA did not inhibit PMA or TNF-alpha-dependent activation of nuclear factor-kB or activation protein-1 in U1 cells. Cell-associated **HIV** protein synthesis also was not decreased by pro-uPA, although the release of virion-associated reverse transcriptase activity was substantially inhibited, suggesting a functional analogy between pro-uPA and the antiviral effects of IFNs. Indeed, cell disruption reversed the inhibitory effect of pro-uPA on activated U1 cells, and ultrastructural analysis confirmed that virions were preferentially retained within cell vacuoles in pro-uPA treated cells. Neither expression of endogenous IFNs nor activation of the IFN-inducible Janus kinase/signal transducer and activator of transcription pathway were induced by pro-uPA. Pro-uPA also inhibited acute **HIV** replication in monocyte-derived macrophages and activated peripheral blood mononuclear cells, although with great inter-donor variability. However, pro-uPA inhibited **HIV** replication in acutely infected promonocytic U937 cells and in ex vivo cultures of lymphoid tissue infected in vitro. Because these effects occurred at concentrations substantially lower than those affecting thrombolysis, pro-uPA may represent a previously uncharacterized class of antiviral agents mimicking IFNs in their inhibitory effects on **HIV** expression and replication.

L16 ANSWER 5 OF 26 MEDLINE on STN

2002240034. PubMed ID: 11967123. Decreased **urokinase** receptor expression on granulocytes in **HIV**-infected patients. Storgaard M; Obel N; Black F T; Moller B K. (Department of Infectious Diseases, Skejby University

Hospital, Denmark.. m.stolgaard@dadlnet.dk) . Scandinavian journal of immunology, (2002 Apr) 55 (4) 409-13. Journal code: 0323767. ISSN: 0300-9475. Pub. country: England: United Kingdom. Language: English.

AB Pericellular proteolysis initiated by receptor-bound **urokinase**-type plasminogen activator (uPA) is considered important for directed migration of granulocytes to inflammatory sites. Using flow cytometry and whole-cell binding of radiolabelled-uPA, we found a high level of uPA-receptor (uPAR) expression in granulocytes (3.9 x 104 +/- 0.9 x 104 sites/cell). Modulation of uPAR expression was assessed in the presence of chemoattractant gradients. Our findings demonstrate that interleukin (IL)-8, leukotriene B4(LTB4) and formyl-methionyl-leucyl-phenylalanine (f MLP) caused a dose-dependent upregulation of uPAR on granulocytes in healthy controls. Modulation of uPAR expression is known to regulate chemotactic response. As determined by flow cytometry, uPAR expression by granulocytes from **human immunodeficiency virus** (**HIV**)-infected patients was distinctly lower than that of healthy control cells ($P < 0.001$). However, upregulation of uPAR in response to chemoattractants was similar to that observed in healthy controls. In **HIV**-infected patients, the uPAR expression on granulocytes correlated ($P < 0.001$, $n = 10$) with the number of CD4+ blood cells. In contrast, the expression of IL-8 receptor, CD11b, CD18 and CD62 was not significantly altered in **HIV**-patients compared with healthy controls.

L16 ANSWER 6 OF 26 MEDLINE on STN

2002173337. PubMed ID: 11906036. **Urokinase** plasminogen activator and TGF-beta production in immunosuppressed patients with and without Pneumocystis carinii. Angelici E; Contini C; Carfagna P; Romani R; Magno M S; Serra P; Canipari R. (Department of Clinical Medicine, University La Sapienza, Rome, Italy.. Elena.angelici@uniroma1.it) . Journal of eukaryotic microbiology, (2001) Suppl 150S-151S. Journal code: 9306405. ISSN: 1066-5234. Pub. country: United States. Language: English.

L16 ANSWER 7 OF 26 MEDLINE on STN

2001347761. PubMed ID: 11409912. Exogenous fibroblast growth factor-2 induces a transformed phenotype in vascular kaposi's sarcoma-like cells. Cavallaro U; Soria M R; Montesano R. (Dibit, Scientific Institute San Raffaele, Milan, Italy.. cavallaro@nt.imp.univie.ac.at) . Molecular cell biology research communications : MCBRC, (2000 Oct) 4 (4) 203-5. Journal code: 100889076. ISSN: 1522-4724. Pub. country: United States. Language: English.

AB Vascular TTB cells derive from murine Kaposi's sarcoma-like dermal lesions and share several phenotypic features with AIDS-associated KS spindle cells. We have recently reported that fibroblast growth factor-2 (FGF-2) promotes dramatic cytoskeletal and morphological alterations in TTB cells, concomitant with the induction of an autocrine loop for hepatocyte growth factor and a relocalization of the **urokinase** receptor. Since all these alterations are hallmarks of cell transformation. we attempted to verify whether FGF-2 induces a transformed phenotype in TTB cells. Our results show that FGF-2-treated TTB cells acquire the ability to grow under anchorage-independent conditions. In addition, FGF-2 markedly reduced the levels of thrombospondin-1, an antiangiogenic and tumor suppressor protein, in TTB cells. Therefore, FGF-2 induces KS-like spindle cells to acquire properties characteristic of transformed cells. This suggests that FGF-2 plays a pathogenetic role in KS not only by promoting angiogenesis, but also by conferring a transformed phenotype upon KS cells. In light of previous reports on Tat-induced release of FGF-2 into the extracellular space, our findings may provide an additional mechanism for the observed synergism between Tat and FGF-2 in the pathogenesis of KS.

L16 ANSWER 8 OF 26 MEDLINE on STN

2001327647. PubMed ID: 11394884. Amino-terminal fragment of **urokinase**-type plasminogen activator inhibits **HIV**-1 replication. Wada M; Wada N A; Shirono H; Taniguchi K; Tsuchie H; Koga J. (Laboratories for Bioengineering and Research, JCR Pharmaceuticals Company, Ltd., 2-2-10

Matsuoka, Nishi-ku, Kobe, 651-2241, Japan.. wada@mejiphalm.co.jp) . Biochemical and biophysical research communications, (2001 Jun 8) 284 (2) 346-51. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB CD8+ T lymphocytes have been shown to produce unidentified soluble factors active in suppressing **HIV**-1 replication. In this study, we purified an **HIV**-1 suppressing activity from the culture supernatant of an immortalized CD8+ T cell clone, derived from an **HIV**-1 infected long-term nonprogressor, and identified this activity as the amino-terminal fragment (ATF) of **urokinase**-type plasminogen activator (uPA). ATF is catalytically inactive, but suppresses the release of viral particles from the **HIV**-1 infected cell lines via binding to its receptor CD87. In contrast, cell proliferation and the secretion of an **HIV**-1 LTR driven reporter gene product were not affected by ATF. These findings suggest that ATF may inhibit the assembly and budding of **HIV**-1, which provides a novel therapeutic strategy for AIDS.

L16 ANSWER 9 OF 26 MEDLINE on STN

2001085920. PubMed ID: 11110678. Serum level of soluble **urokinase**-type plasminogen activator receptor is a strong and independent predictor of survival in **human immunodeficiency virus** infection. Sidenius N; Sier C F; Ullum H; Pedersen B K; Lepri A C; Blasi F; Eugen-Olsen J. (Department of Molecular Pathology and Medicine, Molecular Genetics Unit, DIBIT, San Raffaele Scientific Institute, Milan, Italy.. nicolai.sidenius@hsr.it) . Blood, (2000 Dec 15) 96 (13) 4091-5. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB **Human immunodeficiency virus**-1 (**HIV**-1) infection has been shown to result in up-regulation of the **urokinase**-type plasminogen activator receptor (uPAR/CD87) on leukocytes in vitro and in vivo. The objective of this study was to investigate whether this up-regulation is paralleled by higher serum levels of soluble uPAR (suPAR) in patients with advanced **HIV**-1 disease and whether the serum level of suPAR is predictive of clinical outcome. Using an enzyme-linked immunosorbent assay, the level of suPAR was measured retrospectively in serum samples from 314 patients with **HIV**-1 infection. By Kaplan-Meier and Cox regression analyses, the serum suPAR levels were correlated to survival with AIDS-related death as the end point. High levels of serum suPAR (greater than median) were associated with poor overall survival, and Kaplan-Meier analysis on patients stratified by suPAR level demonstrated a continuous increase in mortality rates with higher suPAR levels. After adjustment for accepted prognostic markers-including Centers for Disease Control and Prevention-defined clinical stages, CD4 counts, viral load, beta2-microglobulin, and age-the prognostic strength of suPAR remained highly significant, indicating that the serum suPAR level is a novel, strong, and independent predictor of survival in **HIV**-1 infection. This report is the first to demonstrate an important association between the plasminogen activator system and disease progression in **HIV**-1 infection.

L16 ANSWER 10 OF 26 MEDLINE on STN

2000182630. PubMed ID: 10722370. Possible role of the plasminogen receptor as a site of interaction of the **human immunodeficiency virus** p24 immunosuppressive heptapeptide Ch7 with the host immune system. Giacomini E; Chersi A; Giordani L; Luzzati A L. (Department of Immunology, Istituto Superiore di Sanita, Rome, Italy.) Scandinavian journal of immunology, (2000 Feb) 51 (2) 164-7. Journal code: 0323767. ISSN: 0300-9475. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Previous work from our laboratory demonstrated that a synthetic heptapeptide (Ch7: RGSDIAG), corresponding to a conserved sequence of **human immunodeficiency virus** (**HIV**) core protein p24 (amino acids 232- 238), was able to specifically abrogate antigen-induced responses in cultures of normal human peripheral blood mononuclear cells (PBMC), probably acting at the level of monocytes. The Ch7 peptide displays sequence homology to human plasminogen. In the present report we show that a compound (6-aminoexanoic acid), known to prevent plasminogen binding to monocyte-like cells, greatly reduced the immunosuppressive

capacity of CM7. We suggest that the plasminogen receptor may represent a target structure on human monocytes for the immunosuppressive p24 sequence.

L16 ANSWER 11 OF 26 MEDLINE on STN

1998383420. PubMed ID: 9717675. **Urokinase** plasminogen activator receptor (uPAR; CD87) expression on monocytic cells and T cells is modulated by **HIV**-1 infection. Speth C; Pichler I; Stockl G; Mair M; Dierich M P. (Institute of Hygiene, University of Innsbruck, Austria.. cornelia.speth@uibk.ac.at) . Immunobiology, (1998 Jul) 199 (1) 152-62. Journal code: 8002742. ISSN: 0171-2985. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB The transmembranous **urokinase**-type plasminogen activator receptor (uPAR; CD87) focuses the formation of active plasmin at the cell surface, thus enhancing directional extracellular proteolysis. Since proteolysis is involved in processes like adhesion, chemotaxis and migration which are important for viral spreading, we investigated the expression of uPAR in **HIV**-infected cells. Expression of CD87 was upmodulated in U937 monocytic cells as well as in the T cell line H9 and in peripheral blood mononuclear cells (PBMC), both on protein and on mRNA level. This upmodulation was not caused by enhanced mRNA stability but by an enhanced transcriptional rate of the CD87 gene as shown by nuclear run-on analysis. To identify the **HIV**-responsive element in the CD87 promoter we investigated the promoter activity in U937 and H9 cells at different time points after **HIV**-infection. Although the transcription of the CD87 gene is higher in **HIV**-infected cells the promoter activity declines after infection, indicating the presence of an additional regulatory element located upstream of the known promoter sequence or in intron sequences.

L16 ANSWER 12 OF 26 MEDLINE on STN

1998380991. PubMed ID: 9715259. Altered levels of **urokinase** on monocytes and in serum of children with AIDS; effects on lymphocyte activation and surface marker expression. Murali R; Wolfe J H; Erber R; Chice S M; Murali M R; Durkin H G; Zach P; Auci D L. (Department of Pathology, State University of New York Health Science Center at Brooklyn, NY 11203, USA.) Journal of leukocyte biology, (1998 Aug) 64 (2) 198-202. Journal code: 8405628. ISSN: 0741-5400. Pub. country: United States. Language: English.

AB **Urokinase** (UK) type plasminogen activator is a serine protease produced by activated human monocytes. Despite the well-documented roles played by UK in cell-mediated immunity in healthy humans, the roles played by UK in the derangements of cell-mediated immune responses observed in **HIV** disease remain largely undefined. In these studies the numbers of peripheral blood lymphocytes and monocytes bearing surface UK (UK+) as well as serum levels of UK (flow microfluorimetry and ELISA, respectively) were determined in children with AIDS and in healthy **HIV**-negative children. The effects of exogenous UK on lymphocyte activation (cell cycle analysis using living cells) and surface marker (CD3, CD4, CD8, and CD19) expression (flow microfluorimetry using fixed cells) were also studied. Data are expressed as percent total cells. Numbers of UK+ lymphocytes in children with AIDS were similar to those observed in healthy children. In contrast, numbers of UK+ peripheral blood monocytes were dramatically decreased (> 70%) in the children with AIDS. However, serum levels of UK were increased (nearly threefold) in these children. When lymphocytes from these children were cultured with soluble UK, numbers of cells in S phase of cell cycle appeared suppressed. Incubation of fixed lymphocytes from either a child with AIDS or from a healthy child with exogenous UK appeared to increase numbers of cells expressing CD3. Incubation with UK had no effect on expression of any other surface marker (CD4, CD8, or CD19) using cells from the child with AIDS. In contrast, incubation with UK appeared to decrease (fivefold) numbers of cells expressing CD19 and increase numbers of cells expressing CD4 and CD8 only when fixed lymphocytes from a healthy **HIV**-negative child were used. The results suggest important roles for UK in regulation of lymphocyte surface markers in general and in CD3- and CD19-dependent lymphocyte activation pathways specifically. Furthermore, these studies add to a widening body of evidence implicating UK dysregulation in the pathogenesis of **HIV**

disease and may point to pharmacological opportunities involving uR to delay or prevent progression of **HIV** infection into full-blown AIDS.

L16 ANSWER 13 OF 26 MEDLINE on STN

1998370672. PubMed ID: 9707175. FGF-2 stimulates migration of Kaposi's sarcoma-like vascular cells by HGF-dependent relocalization of the **urokinase** receptor. Cavallaro U; Wu Z; Di Palo A; Montesano R; Pepper M S; Maier J A; Soria M R. (Department of Biological and Technological Research, San Raffaele Scientific Institute, Milano, Italy.. cavallu@dibit.hsr.it) . FASEB journal : official publication of the Federation of American Societies for Experimental Biology, (1998 Aug) 12 (11) 1027-34. Journal code: 8804484. ISSN: 0892-6638. Pub. country: United States. Language: English.

AB The spindle-shaped cell line TTB was recently isolated from highly vascularized skin lesions of BKV/**HIV**-1 tat transgenic mice and shown to possess an autocrine loop for hepatocyte growth factor (HGF). We show that fibroblast growth factor-2 (FGF-2) stimulates TTB cell migration and promotes polarization of uPAR at the leading edge of migrating cells. FGF-stimulated TTB cells presented the typical migratory phenotype, with a triangular cell shape and concomitant breakdown of actin stress fibers and smooth muscle-specific actin isoform. FGF-2-stimulated migration was blocked by antibodies against **urokinase**-type plasminogen activator (uPA) or uPA receptor (uPAR) and by neutralizing anti-HGF antibodies. The latter also inhibited uPAR relocalization at the cell surface of FGF-2-treated TTB cells. This points to a crosstalk between FGF-2 and HGF that might mediate TTB cell migration by modulating the localization of cell surface uPAR.

L16 ANSWER 14 OF 26 MEDLINE on STN

97383256. PubMed ID: 9236217. Glutamate-dependent activation of NF-kappaB during mouse cerebellum development. Guerrini L; Molteni A; Wirth T; Kistler B; Blasi F. (Department of Genetics and Microbial Biology, University of Milan, 20133 Milan, Italy.) Journal of neuroscience : official journal of the Society for Neuroscience, (1997 Aug 15) 17 (16) 6057-63. Journal code: 8102140. ISSN: 0270-6474. Pub. country: United States. Language: English.

AB NF-kappaB and activator protein 1 (AP-1) are dimeric transcription factors involved in transcriptional regulation in many cells, including neurons. We have examined their activity during mouse cerebellum development, a postnatal process starting just after birth and completed by the fourth postnatal (PN) week. The activity of these factors was analyzed by binding of nuclear extracts to a synthetic oligonucleotide representing the kappaB site of **human immunodeficiency virus** or the AP-1 site of the **urokinase** promoter. NF-kappaB activity was observed from 7 PN, was restricted to the developing cerebellum, and was not observed in the early postnatal neocortex and hippocampus. On the other hand, AP-1 activity was not found in cerebellum but was present in both neocortex and hippocampus. Moreover, a kappaB-driven transgene was found to be increasingly expressed in the cerebellum from 5 PN to 10 PN but not in the adult. The regulation of NF-kappaB activation in mouse cerebellum was analyzed by intraperitoneal injection of glutamate receptor antagonists to 9 PN mice, which abolished NF-kappaB-binding activity, suggesting an endogenous loop of glutamate receptor activation. Glutamate receptor agonists, on the other hand, induced NF-kappaB nuclear translocation in the cerebellum of 5 PN mice, which is a stage in which NF-kappaB is not yet endogenously activated. This effect was specific for NF-kappaB and not observed for AP-1. In adult mice, NF-kappaB activity was absent in the cerebellum and was not induced by intraperitoneal injection of glutamate receptor agonists. These data show that NF-kappaB is specifically activated during cerebellum development and indicate an important role of glutamate receptors in this process.

L16 ANSWER 15 OF 26 MEDLINE on STN

97288653. PubMed ID: 9143604. Upregulation of **urokinase**-type plasminogen activator by endogenous and exogenous **HIV**-1 Tat protein in tumour cell lines derived from BK virus/tat-transgenic mice. Rusnati M; Coltrini D;

Campioni D; Tanghetti E; Corallini A; Balbanti Brodano G; Giuliani R; Gibellini D; Presta M. (Department of Biomedical Sciences and Biotechnology, University of Brescia, Italy.) AIDS (London, England), (1997 May) 11 (6) 727-36. Journal code: 8710219. ISSN: 0269-9370. Pub. country: United States. Language: English.

AB OBJECTIVE: To demonstrate that Tat modulates the plasminogen-dependent proteolytic activity of tumour cell lines derived from BK virus (BKV)/tat-transgenic mice by affecting the production of plasminogen activators (PA) and the PA inhibitor (PAI)-1 and to demonstrate that this occurs through mechanism(s) that are distinct from those responsible for transactivating activity of extracellular Tat. DESIGN AND METHODS: To assess whether endogenous Tat is responsible for PA activity in T53 adenocarcinoma cells, cell cultures were transfected with antisense Tat cDNA and evaluated for cell-associated PA activity by a plasmin chromogenic assay. The assay was also used to evaluate PA activity in T53 cells and T111 leiomyosarcoma cells stimulated by extracellular Tat. The type(s) of PA produced were identified by sodium dodecyl sulphate-polyacrylamide gel electrophoresis zymography. The levels of PAI-1 were evaluated by Western blotting. Tat transactivating activity was measured by a chloramphenicol acetyltransferase (CAT) enzyme-linked immunosorbent assay in HL3T1 cells containing integrated copies of an **HIV**-1 long terminal repeat (LTR)-CAT plasmid. RESULTS: Transfection of T53 cells with antisense Tat cDNA results in the decrease of Tat production and PA activity. Exogenously added Tat increases PA levels in T53 and in T111 cells. PA activity was identified as **urokinase**-type PA (uPA). Tat also increases the production of PAI-1 in T111 but not in T53 cells. Chloroquine and heparin have different affects on the LTR-CAT-transactivating and the PA-inducing activities of Tat. The fusion protein glutathione-S-transferase-Tat and the mutant Tat-1e, lacking the second Tat exon, cause LTR-CAT transactivation without stimulating uPA upregulation. CONCLUSIONS: Tat affects the fibrinolytic activity of tumour cell lines derived from BKV/tat-transgenic mice by modulating the production of both uPA and PAI-1 via autocrine and paracrine mechanisms of action. The capacity of Tat to modulate the plasminogen-dependent proteolytic activity of these tumour cell lines may contribute to their metastatic potential. The uPA-inducing activity of Tat depends upon specific biological and structural features of the Tat protein that are distinct from those responsible for its LTR-CAT-transactivating activity, suggesting distinct mechanisms of induction for the two biological responses.

L16 ANSWER 16 OF 26 MEDLINE on STN

97037021. PubMed ID: 8882668. Production of plasminogen activator and plasminogen activator inhibitors by alveolar macrophages in control subjects and AIDS patients. Angelici E; Contini C; Romani R; Epifano O; Serra P; Canipari R. (Department of Clinical Medicine, La Sapienza University, Rome, Italy.) AIDS (London, England), (1996 Mar) 10 (3) 283-90. Journal code: 8710219. ISSN: 0269-9370. Pub. country: United States. Language: English.

AB OBJECTIVE: To reveal a possible impairment of the plasminogen activator system in the pulmonary infections of AIDS patients. DESIGN: To test the plasminogen activator system functionality in alveolar macrophages and bronchoalveolar lavage fluid (BALF) in control subjects and AIDS patients. Procedures were designed to detect the presence of imbalance in plasminogen activator activity and to ascertain if this imbalance is due to a direct effect of the **HIV** virus on macrophages or to superimposed opportunistic infection. METHODS: Alveolar macrophages obtained by bronchoalveolar lavage (BAL) were either lysed with Triton X-100 or cultured for 24 h. Plasminogen activators and plasminogen activator inhibitors (PAI) were measured by chromogenic substrate assay and binding to 125I-**urokinase** followed by 10% sodium dodecyl-sulphate polyacrylamide gel electrophoresis (SDS-PAGE), respectively. RESULTS: Plasminogen activator activity in BALF and in alveolar macrophages from AIDS patients was decreased. This reduction was independent of the presence of an infectious pulmonary process. In contrast, free PAI was increased in AIDS patients with Pneumocystis carinii infection. This increase is possibly

caused by a different glycosylated form of PAI-2. CONCLUSIONS: Our data support the view that the pulmonary fibrogenic response is in part secondary to an imbalance within the plasminogen activator system and provide the basis for clarifying the role of these alterations in the pathophysiology of AIDS-related pulmonary infections.

L16 ANSWER 17 OF 26 MEDLINE on STN

96258627. PubMed ID: 8778349. [Synthetic inhibitors targeting serine and aspartic acid proteases]. Inhibiteurs synthetiques a visee pharmacologique ciblant les proteases a serine et a acides aspartiques. Reboud-Ravaux M C; Boggetto N D; Doucet C E; de Rosny E H; Vergely I B; Thierry N M; Amour A J. (Departement de Biologie Supramoleculaire et Cellulaire, Institut Jacques Monod, Universite de Paris VII, France.. reboud@ccr.jussieu.fr) . Journal de pharmacie de Belgique, (1996 May-Jun) 51 (3) 161-4. Ref: 44. Journal code: 0375351. ISSN: 0047-2166. Pub. country: Belgium. Language: French.

AB The interaction of novel series of synthetic inhibitors with various serine proteases (leukocyte elastase, thrombin, cathepsin G, chymotrypsin, plasminogen activators and plasmin) and an aspartic protease (**HIV**-1 protease) were studied. Various aspects were analyzed: mechanism of action, structure-activity relationships, and in some cases, molecular modelling and biological evaluation. Functionalized cyclopeptides and N-aryl azetidin-2-ones behaved as suicide substrates acting specifically on trypsin-like proteases (thrombin or **urokinase**) and elastases, respectively. Novel hydrazinopeptides acted as reversible inhibitors of elastases. Coumarin derivatives inactivated very efficiently chymotrypsin-like proteases (k(inact)/K(I) = 760,000 M(-1) .s(-1)). Inhibitors of **HIV**-1 protease acting either as inactivators or dimerization inhibitors are under investigation. The inhibitors described above are useful for elucidating the biological roles of the target enzymes and constitute potential drugs.

L16 ANSWER 18 OF 26 MEDLINE on STN

96256755. PubMed ID: 8676469. A role for **urokinase**-type plasminogen activator in **human immunodeficiency virus** type 1 infection of macrophages. Handley M A; Steigbigel R T; Morrison S A. (Department of Pharmacology, University Medical Center at Stony Brook, Stony Brook, New York, USA.) Journal of virology, (1996 Jul) 70 (7) 4451-6. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB **Urokinase**-type plasminogen activator (uPA), a proteinase which activates plasminogen by cleaving at -CPGR(arrow downward)V-, was shown to cleave the V3 loop in recombinant gp120 of **human immunodeficiency virus** type 1 (**HIV**-1) IIIB and MN strains, as well as a synthetic, cyclized peptide representing the clade B consensus sequence of V3. Proteolysis occurred at the homologous -GPGR(arrow downward)A-, an important neutralizing determinant of **HIV**-1. It required soluble CD4 and was prevented by inhibitors of uPA but not by inhibitors of likely contaminating plasma proteinases. It was accelerated by heparin, a known cofactor for plasminogen activation. In immune capture experiments, tight binding of uPA to viral particles, which did not depend on CD4, was also demonstrated. Active site-directed inhibitors or uPA diminished this binding, as did a neutralizing antibody to V3. Addition of exogenous uPA to the laboratory-adapted IIIB strain of **HIV**-1, the macrophage-tropic field strains JR-CSF and SF-162, or a fresh patient isolate of indeterminate tropism, followed by infection of macrophages with the various treated viruses, resulted in severalfold increases in subsequent viral replication, as judged by yields of reverse transcriptase activity and p24 antigen, as well as incorporation, as judged by PCR in situ. These responses were reversible by inhibitors or antibodies targeting the proteinase active site or the V3 loop. We propose that uPA, a transcriptionally regulated proteinase which is upregulated when macrophages are **HIV** infected, can be bound and utilized by the virus to aid in fusion and may be an endogenous component that is critical to the infection of macrophages by **HIV**-1.

L16 ANSWER 19 OF 26 MEDLINE on STN

96152765. PubMed ID: 8567112. Over expression of hepatocyte growth factor in human Kaposi's sarcoma. Maier J A; Mariotti M; Albini A; Comi P; Prat M; Comoglio P M; Soria M R. (Department of Biological and Technological Research-Dibit, San Raffaele Institute, Milan, Italy.) International journal of cancer. Journal international du cancer, (1996 Jan 17) 65 (2) 168-72. Journal code: 0042124. ISSN: 0020-7136. Pub. country: United States. Language: English.

AB Kaposi's sarcoma is a highly vascularized multifocal tumor which frequently appears as a complication of **HIV** infection. It has been suggested that a disorder in the cytokine network may contribute to the development of the disease. We examined the expression of several cytokines in human sporadic Kaposi's-sarcoma specimens, as well as in spindle cells cultured from human lesions, and consistently found high levels of expression of hepatocyte growth factor (HGF). In addition, human lesion-derived spindle cells synthesize and secrete biologically active hepatocyte growth factor and express the hepatocyte-growth-factor receptor (c-MET). Moreover, elevated levels of transforming growth factor beta 1 (TGF beta 1) mRNA were found in lesions of human sporadic Kaposi's sarcoma and in lesion-derived spindle cells which also over-express **urokinase**. Since HGF, TGF beta 1 and **urokinase** are all involved in capillary-vessel organization, dysregulation of these interacting agents may play a role in the initiation and/or progression of Kaposi's sarcoma, stimulating the growth of spindle cells and recruiting endothelial cells into the lesion.

L16 ANSWER 20 OF 26 MEDLINE on STN

94350977. PubMed ID: 8071349. Differential DNA sequence specificity and regulation of **HIV**-1 enhancer activity by cRel-RelA transcription factor. Hansen S K; Guerrini L; Blasi F. (Department of Genetics and Microbiol Biology, University of Milano, Italy.) Journal of biological chemistry, (1994 Sep 2) 269 (35) 22230-7. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The cRel-RelA and NF-kappa B (p50-RelA) transcription factors bind to a kappa B-like sequence termed Rel-related proteins binding element localized in the regulatory region of the **human urokinase plasminogen activator** (uPA) gene. This sequence is highly conserved in murine and porcine uPA genes where it retained the ability to associate with cRel-RelA. On the other hand, NF-kappa B binding was obtained with the human and porcine elements only. Methylation interference analysis showed that NF-kappa B and cRel-RelA had identical interference patterns. Mutational analysis showed that DNA binding was highly sensitive to mutations within the decameric Rel-related proteins binding element core site. However, alterations of nucleotides flanking the decameric IgK-kappa B motif, which preferentially associated with NF-kappa B, resulted in high affinity cRel-RelA binding both in vitro and in vivo. These data demonstrate that NF-kappa B and cRel-RelA have overlapping but distinct DNA sequence specificities. Bandshift analysis with HeLa and Jurkat cell extracts or with in vitro translated proteins revealed that the SV40-, **HIV**-1-, and interleukin-2 receptor alpha subunit kappa B elements efficiently associated with cRel-RelA, suggesting that this heterodimer may be involved in the regulation of several genes. Cotransfection studies of **HIV**-1 long terminal repeat-chloramphenicol acetyltransferase reporter DNA with RelA, cRel, and p50 expression vectors were performed in COS7 and U293 cells to analyze the ability of cRel-RelA to regulate **HIV**-1 enhancer activity. In vivo formation of the cRel-RelA complex resulted in specific stimulation of the viral enhancer at a level comparable with that obtained with NF-kappa B. These data suggest that activation of cellular cRel-RelA may play a critical role in the regulation of **HIV**-1 enhancer activity.

L16 ANSWER 21 OF 26 MEDLINE on STN

94338592. PubMed ID: 7520247. Characterization of a human Kaposi's sarcoma cell line that induces angiogenic tumors in animals. Herndier B G; Werner A; Arnstein P; Abbey N W; Demartis F; Cohen R L; Shuman M A; Levy J A. (Department of Pathology, San Francisco General Hospital, California.) AIDS (London, England), (1994 May) 8 (5) 575-81. Journal code: 8710219.

ISSN: 0269-9370. Pub. country: United States. Language: English.
AB OBJECTIVE: To characterize a Kaposi's sarcoma (KS) cell line established from a tumor biopsy from the oral mucosa of an iatrogenically immunosuppressed **HIV**-negative man. METHODS: Cells were placed in culture and evaluated by a variety of biologic, serologic, karyotypic, and immunologic procedures. Electron microscopic examination was performed. The ability to produce tumors in nude mice was evaluated, and the nature of the cells within the tumor determined. Assays for **urokinase** plasminogen activator type (uPA), plasminogen activator inhibitor-1 (PAI-1) and the **urokinase** receptor (uPAR) were conducted. RESULTS: The SLK cell line has an endothelial cell morphology with very little anaplasia. The karyotype indicates diploid phenotype of human origin. Immunohistochemical and electron microscopic examinations confirmed the endothelial nature of this cell line. No viruses were detected. The tumors induced in nude mice showed hypervascularization, with characteristics of KS. The cell line produces uPA and PAI-1, and also expresses uPAR. CONCLUSIONS: The SLK cell line is of endothelial cell origin and the first human cell line to induce KS-like tumors in recipient animals. The expression of **urokinase** and its receptor suggests a paracrine and autocrine interaction that may be important for the growth of the tumor. The SLK line should be valuable for studies of KS pathogenesis and therapeutic approaches to this malignancy.

L16 ANSWER 22 OF 26 MEDLINE on STN
94110605. PubMed ID: 8283034. **Urokinase** receptor. An activation antigen in human T lymphocytes. Nykjaer A; Moller B; Todd R F 3rd; Christensen T; Andreasen P A; Gliemann J; Petersen C M. (Institute of Medical Biochemistry, University of Aarhus, Denmark.) Journal of immunology (Baltimore, Md. : 1950), (1994 Jan 15) 152 (2) 505-16. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United States. Language: English.
AB The ability of activated T lymphocytes to extravasate and reach inflammatory and malignant foci in the tissues is a basic function of cellular immunity. Recent evidence strongly suggests that the **urokinase** receptor (uPAR) holds a central position in the development of human two-chain **urokinase**-mediated pericellular proteolysis and matrix degradation, an important element in cell migration. In this report we establish uPAR as a pan T cell activation Ag. As determined by FACS analysis, CD3+ lymphocytes from healthy donors exhibited no significant uPAR expression. In contrast, patients (e.g., **HIV**-positive donors) showed distinct uPAR expression, confined to HLA-DR+ cells, in up to 80% of all T cells. In vitro activation by PMA caused a rapid up-regulation of membrane uPAR in all healthy donor T cells and was accompanied by enhanced receptor synthesis and elevated uPAR mRNA levels. A similar induction resulted from activation via the TCR/CD3 complex using mitogens (PHA, and Con A), anti-CD3 antibodies, and alloantigen. Receptor expression at single cell level was also modulated by a number of cytokines. IL-2, IL-4 and IL-7 increased uPAR presentation on 20 to 50% of the T cell population, and combined stimulation of bulk cultures demonstrated an additive effect of IL-2 and IL-7, whereas the response to each of the two was inhibited by IL-4. In addition, TGF-beta 1 substantially reduced the uPAR expression in T cell cultures responding to PHA, IL-2, and IL-7. Irrespective of the activating reagent, the T cells appeared to produce the same molecular uPAR species, but the affinity of uPAR expressed in PMA blasts was decreased, presumably because of a differential location at the cell surface. All activated cultures showed co-expression of uPAR and CD25. The finding that the **urokinase** receptor is an activation Ag may suggest that cell-associated plasminogen activation is involved in extravasation and migration of activated T cells.

L16 ANSWER 23 OF 26 MEDLINE on STN
94047305. PubMed ID: 8230418. Effects of the tat and nef gene products of **human immunodeficiency virus** type 1 (**HIV**-1) on transcription controlled by the **HIV**-1 long terminal repeat and on cell growth in macrophages. Murphy K M; Sweet M J; Ross I L; Hume D A. (Centre for Molecular Biology and Biotechnology, University of Queensland, Australia.

, Journal of Virology, (1993 Dec) 67 (12) 6956-64. Journal code: 0113724. ISSN: 0022-538X. Pub. country: United States. Language: English.

AB The RAW264 murine macrophage cell line was used as a model to examine the role of the tat and nef gene products in the transcription regulation of the **human immunodeficiency virus** type 1 (**HIV**-1) long terminal repeat (LTR) in macrophages. Contrary to claims that the activity of the **HIV**-1 LTR responds poorly in rodent cells to trans activation by the viral tat gene product, cotransfection of RAW264 cells with a tat expression plasmid in transient transfection assays caused a > 20-fold increase in reporter gene expression that was inhibited by mutations in the TAR region. RAW264 cells stably transfected with the tat plasmid displayed similarly elevated **HIV**-1 LTR-driven reporter gene activity. By contrast to previous reports indicating a negative role for nef in **HIV** transcription, cotransfection of RAW264 cells with a nef expression plasmid trans activated the **HIV**-1 LTR driving either a chloramphenicol acetyltransferase or a luciferase reporter gene. The action of nef was specific to the LTR, as expression of nef had no effect on the activity of the simian virus 40, c-fms, **urokinase** plasminogen activator, or type 5 acid phosphatase promoter. trans-activating activity was also manifested by a frameshift mutant expressing only the first 35 amino acids of the protein. The effects of nef were multiplicative with those of tat gene product and occurred even in the presence of bacterial lipopolysaccharide, which itself activated LTR-directed transcription. Examination of the effects of selected mutations in the LTR revealed that neither the kappa B sites in the direct repeat enhancer nor the TAR region was required as a cis-acting element in nef action. The action of nef was not species restricted; it was able to trans activate in the human monocyte-like cell line Mono Mac 6. The presence of a nef expression cassette in a neomycin phosphotransferase gene expression plasmid greatly reduced the number of G418-resistant colonies generated in stable transfection of RAW264 cells, and many of the colonies that were formed exhibited very slow growth. The frameshift mutant was also active in reducing colony generation. Given the absence of any effect of the frameshift mutation on nef function, its actions on macrophage growth and **HIV** transcription are discussed in terms of the role of the N-terminal 30 amino acids and of stable secondary structures in the mRNA.

L16 ANSWER 24 OF 26 MEDLINE on STN

92395377. PubMed ID: 1522387. Constitutive production of PAI-II and increased surface expression of GM1 ganglioside by peripheral blood monocytes from patients with AIDS: evidence of monocyte activation in vivo. Auci D L; Chice S M; Durkin H G; Murali M R. (Department of Pathology, State University of New York Health Science Center, Brooklyn 11203.) Journal of leukocyte biology, (1992 Sep) 52 (3) 282-6. Journal code: 8405628. ISSN: 0741-5400. Pub. country: United States. Language: English.

AB To characterize the activation state of monocytes during **human immunodeficiency virus** (**HIV**) infection, peripheral blood monocytes (PBMs) from patients with acquired immunodeficiency syndrome (n = 10) and from healthy controls (n = 10) were cultured for 4 days. Monocyte culture supernatant (MCS) was collected daily, and levels of **urokinase** (UK) inhibitor PAI-II, a product of activated monocytes, released into MCS were determined (fibrin plate assay). To examine the activation state of PBMs independently, expression of GM1 ganglioside on PBMs from patients with AIDS (n = 9), patients with AIDS-related complex (ARC) (n = 8), **HIV**+ asymptomatic patients (n = 6), and **HIV**- healthy controls (n = 11) was determined (flow cytometry; living cells in suspension). Data are expressed as percent inhibition of UK, or as percent total cells. Patients' MCS collected on days 1-4 of culture contained similar levels of PAI-II because it inhibited UK in similar fashion (70-90%). In contrast, MCS from healthy controls, collected after 2 days, had decreased ability to inhibit UK (15-50%) and thus contained lower levels of PAI-II. Monocyte activation, measured by increased expression of GM1 ganglioside on PBM surfaces, directly correlated with the progression of **HIV** infection into the development of AIDS, since the order of magnitude of GM1 ganglioside expression on PBMs was AIDS greater than ARC greater than

HIV+ asymptomatic - healthy controls. Our data indicate that PBMs from patients with AIDS are constitutively activated and suggest that activation directly correlates with disease progression.

L16 ANSWER 25 OF 26 MEDLINE on STN
92225083. PubMed ID: 1563500. Procoagulant and fibrinolytic activities in bronchoalveolar fluid of **HIV**-positive and **HIV**-negative patients. De Benedetti E; Nicod L; Reber G; Vifian C; de Moerloose P. (Dept of Medicine, University Cantonal Hospital, Geneva, Switzerland.) European respiratory journal : official journal of the European Society for Clinical Respiratory Physiology, (1992 Apr) 5 (4) 411-7. Journal code: 8803460. ISSN: 0903-1936. Pub. country: Denmark. Language: English.
AB Imbalance between intra-alveolar procoagulant activity (PCA) and fibrinolytic activity may lead to fibrin deposition, as described in several pneumopathies, and may eventually contribute to fibrotic changes as observed in Pneumocystis carinii pneumonia (PCP). The aim of our study was to compare these activities in bronchoalveolar lavages of **human immunodeficiency virus** (**HIV**)-positive and **HIV**-negative patients. The material comprised: a) controls (n = 7); b) **HIV**-positive patients subdivided into PCP (n = 11), bacterial pneumonia (n = 8) and other pneumopathies (n = 22); and c) **HIV**-negative patients with bacterial pneumonia (n = 8). PCA was significantly increased (p less than 0.05) in all patient groups compared to controls. The **urokinase**-type plasminogen activator (u-PA) antigen levels were highest during bacterial pneumonia. Regardless of the **HIV** status, in bacterial pneumonia there was a marked elevation of plasminogen activator inhibitor antigens with little residual fibrinolytic activity. In contrast, the fibrinolytic activity was not decreased in PCP. D-dimer were elevated during PCP compared to controls; the highest levels were found in **HIV**-negative bacterial pneumonia. These data indicate that transient fibrotic changes seen in PCP may be favoured by increased PCA, but not by a depressed fibrinolytic activity. In bacterial pneumonia PCA is increased and fibrinolysis decreased independently of the **HIV** status.

L16 ANSWER 26 OF 26 MEDLINE on STN
90152351. PubMed ID: 2515992. High-level expression of recombinant genes in Escherichia coli is dependent on the availability of the dnaY gene product. Brinkmann U; Mattes R E; Buckel P. (Department of Genetics, Boehringer Mannheim GmbH, Penzberg (F.R.G.).) Gene, (1989 Dec 21) 85 (1) 109-14. Journal code: 7706761. ISSN: 0378-1119. Pub. country: Netherlands. Language: English.
AB We have observed that proteins, such as human tissue-type plasminogen activator, pro-**urokinase** or gp41 of **human immunodeficiency virus**, which have a high content of rare codons in their respective genes, are not readily expressed in Escherichia coli. Furthermore induction of these heterologous genes leads to growth inhibition and plasmid instability. Supplementation with tRNA(AGA/AGG(Arg)) by cotransfection with the dnaY gene, which supplies this minor tRNA, resulted in high-level production with greatly improved cell viability and plasmid stability.

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FILE 'USPATFULL' ENTERED AT 22:24:23 ON 10 MAR 2004
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E WADA NAOKO/IN
L2 2 S E3

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L3 875 S E3
L4 2 S L3 AND (UROKINASE)
E WADA N/AU

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L5              3 S L10

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L7           38 S L6 AND (HUMAN UROKINASE PLASMINOGEN ACTIVATOR)
L8            2 S L7 AND (HUMAN UROKINASE PLASMINOGEN ACTIVATOR/CLM)
L9           36 S L7 NOT L8
L10         166 S L6 AND (ATF OR AMINO-TERMINAL FRAGMENT)
L11          13 S L10 AND (ATF/CLM OR AMINO-TERMINAL FRAGMENT/CLM)

     FILE 'MEDLINE' ENTERED AT 22:38:16 ON 10 MAR 2004
L12        9092 S (UROKINASE OR HUMAN UROKINASE PLASMINOGEN ACTIVATOR)
L13          26 S L12 AND (HUMAN UROKINASE PLASMINOGEN ACTIVATOR)
L14         133 S L12 AND (ATF OR AMINO TERMINAL FRAGMENT)
L15         129 S L14 NOT L13
L16          26 S L12 AND (HIV OR HUMAN IMMUNODEFICIENCY VIRUS)

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